VOLUME 4 May 1955 NUMBER 3

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

Copyright 1955, By The American Society of Tropical Medicine and Hygiene
Made in U.S.A.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Editor-in-Chief, L. W. HACKETT
School of Public Health, University of California
Berkeley 4, California

Editor, Tropical Medicine and Hygiene News
LEON JACOBS, National Institutes of Health
Bethesda 14, Maryland

Editorial Board

G. ROBERT COATNEY, *Chairman*
ERNEST CARROLL FAUST
HAROLD FARNSWORTH GRAY
WILLIAM McD. HAMMON
HERBERT G. JOHNSTONE
EDWIN H. LENNETTE
HARRY MOST
LLOYD E. ROZEBOOM

OFFICERS OF THE
American Society of Tropical Medicine and Hygiene

President
WILLIAM H. TALIAFERRO

President-Elect
ASA C. CHANDLER

Vice-President
DONALD B. McMULLEN

Secretary-Treasurer
JOHN E. LARSH, JR.
School of Public Health
University of North Carolina
Chapel Hill, North Carolina

Councilors:	*Term Expires*
EDWIN H. LENNETTE	1955
G. ROBERT COATNEY	1955
CLAY G. HUFF	1956
SAMUEL W. SIMMONS	1956
ALBERT B. SABIN	1957
J. J. SAPERO	1957
MARSHALL C. BALFOUR	1958
THOMAS H. WELLER	1958

Editor
L. W. HACKETT

Individuals interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care and human welfare are eligible for membership. Dues are $8 a year and include subscription to the American Journal of Tropical Medicine and Hygiene. An application blank is bound into this issue of the Journal for the use of interested individuals. Before being submitted to the Secretary, each application must be endorsed by two active members of the Society and a check for $8 (U. S. banks preferred) must accompany the application.

Entered as second-class matter March 16, 1921, at the Post Office at Baltimore, Maryland, under the act of March 3, 1879.
Subscription price $10.00 per volume. Single numbers, $2.00.

The Society has received an additional supply of the Fiftieth Anniversary Medal. Will those whose orders could not be filled last year please send $3.15 to the Secretary-Treasurer for each medal desired.

This bronze medal, suitable for a paper weight, is 2½ inches in diameter, bears the seal of the present Society on the obverse, and on the reverse the inscription

"FIFTIETH ANNIVERSARY
OF THE FOUNDING OF
THE AMERICAN SOCIETY
OF TROPICAL MEDICINE"

The price will be $3.15, including postage. The slight increase over the price quoted last year is due to increased cost of manufacture. In ordering, please use the form below.

_____, '195

Dr. John E. Larsh, Jr., *Sec'y-Treas.*
School of Public Health
University of North Carolina
Chapel Hill, N. C.

Please send me postpaid, _____ bronze medal(s) commemorating the fiftieth anniversary of the American Society of Tropical Medicine. I enclose check, (money order) for $3.15 for each.

NAME_____

STREET AND NUMBER_____

CITY AND ZONE_____ STATE_____

an ESTABLISHED dosage form
of choice
for PROVED broad-spectrum
antibiotic therapy

TERRAMYCIN®
Brand of oxytetracycline
INTRAMUSCULAR

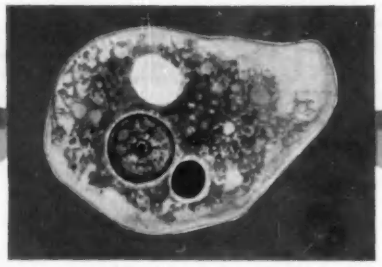
the *first* broad-spectrum antibiotic
available in this convenient parenteral form
for the treatment of a wide range
of infections due to susceptible organisms.

Supplied in single-dose vials. On reconstitution,
each single dose contains: Crystalline
Terramycin hydrochloride—100 mg.
Magnesium chloride—5%
Procaine hydrochloride—2%

Pfizer PFIZER LABORATORIES, *Brooklyn 6, N. Y.*
Division, Chas. Pfizer & Co., Inc.

(In writing to advertisers, please mention the journal—it helps.)

AMEBIASIS:
Intestinal and Extra-Intestinal



Amebiasis may be asymptomatic or may produce symptoms indicating low grade infection, inflammatory colonic disease with or without diarrhea, or symptoms resembling appendicitis, gallbladder disease, or peptic ulcer.[1]

"Frequently an unrecognized hepatitis coexists with intestinal amebiasis."[2]

For complete treatment of amebiasis, combined therapy with Milibis® and Aralen® is highly recommended.

① Intestinal amebic infestation

MILIBIS

—a powerful amebicide, virtually nontoxic and highly effective clinically. Its relative insolubility assures high concentration in the large intestine.

Supplied: 0.5 Gm. tablets – bottles of 25 and 500.

② Extra-intestinal amebiasis

ARALEN PHOSPHATE

—"specific for amebic hepatitis and amebic liver abscess."[2] As effective as emetine but without its toxicity.[3]

Supplied: 0.25 Gm. tablets – bottles of 100 and 1000.

WINTHROP-STEARNS INC.
New York 18, N. Y. • Windsor, Ont.

Milibis and Aralen, trademarks reg. U. S. Pat. Off.,
brand of bismuth glycolylarsanilate and chloroquine, respectively.

1. Sodeman, W. A., and Beaver, P. C.: *Am. Jour. Med.*, 12:440, Apr., 1952.
2. Zavala, D. C., and Hamilton, H. E.: *Ann. Int. Med.*, 36:110, Jan., 1952.
3. Conan, N. J.: *Am. Jour. Med.*, 6:309, Mar., 1949.

WINTHROP

THE AMERICAN JOURNAL OF

TROPICAL MEDICINE AND HYGIENE

Vol. 4 *May, 1955* *No. 3*

CONTENTS

SYMPOSIUM ON SCHISTOSOMIASIS

Introduction to the schistosomiasis symposium. DONALD B. MCMULLEN 381

Biology of the schistosome complexes. ROBERT E. KUNTZ 383

Discussion of the paper by Robert E. Kuntz. GEORGE R. LARUE 414

The natural history and control of the snails that transmit the schistosomes of man. LOUIS OLIVIER 415

Discussion of the paper by L. J. Olivier. HENRY VAN DER SCHALIE 424

The biology and control of the amphibious snails that serve as intermediate hosts for *Schistosoma japonicum*. LAWRENCE S. RITCHIE 426

Discussion of the paper by Lawrence S. Ritchie. H. F. HSÜ 442

Immunological aspects of infections with *Schistosoma mansoni*. JOSÉ OLIVER-GONZÁLEZ, PRESTON M. BAUMAN AND A. S. BENENSON 443

Discussion of Dr. Oliver-González' paper. FREDERIK B. BANG 453

Treatment of schistosomiasis. HARRY MOST 455

Discussion of Dr. Harry Most's report on therapy of schistosomiasis. HAMILTON H. ANDERSON 460

Some contributions of tropical peoples in the preservation of food materials. WILLIAM H. ADOLPH 461

Epidemiology of amebiasis: possible influence of water supply, coincident with diverse features of terrain, on the occurrence of intestinal protozoa. DALE E. WYKOFF, J. R. C. FONSECA AND L. S. RITCHIE 465

The use of penicillin and streptomycin in the routine cultivation of amebae from fecal specimens. LOIS NORMAN AND M. M. BROOKE 472

The effectiveness of the PVA-fixative technique in revealing intestinal amebae in diagnostic cultures. LOIS NORMAN AND M. M. BROOKE 479

Historical evidence for interference between dengue and yellow fever. HARALD FREDERIKSEN 483

Classification of leptospiral isolates from Malaya, Thailand and North Borneo. AARON D. ALEXANDER, PSYCHE W. WETMORE, LARUE B. EVANS, HELEN JEFFRIES AND CHESTER A. GLEISER 492

Treatment of the acute febrile phase of Carrión's disease with chloramphenicol. OSCAR URTEAGA B. AND EUGENE H. PAYNE 507

A survey of poliomyelitis virus antibodies in French Morocco. JOHN R. PAUL AND DOROTHY M. HORSTMANN 512

A survey of forest mosquitoes in relation to sylvan yellow fever in the Panama isthmian area. HAROLD TRAPIDO, PEDRO GALINDO AND STANLEY J. CARPENTER 525

Forest canopy mosquitoes associated with the appearance of sylvan yellow fever in Costa Rica, 1951. PEDRO GALINDO AND HAROLD TRAPIDO 543

Rabies transmitted by bats in British Guiana. B. B. G. NEHAUL 550

An additional species of insectivorous bat naturally infected with rabies. J. E. GRIMES, R. B. EADS AND J. V. IRONS 554

A study of the oviposition activity of three species of *Anopheles* in the laboratory. ROBERT C WALLIS 557

Books received 564

Book reviews: *Histology*, by Greep (Ed.); *Plague*, by Pollitzer; *Medical Mycology*, by Simons (Ed.); *Malnutrition in African Mothers, Infants and Young Children*, Report of 2nd Inter-African Conference on Nutrition; *Diseases of the Skin for Practitioners and Students*, by Andrews; *Histopathologic Technic and Practical Histochemistry*, by Lillie; *Wine as Food and Medicine*, by Lucia; *Care of Laboratory Animals*, American Public Health Association 564

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE is issued bi-monthly, appearing in January, March, May, July, September, and November. One volume a year is issued.

Accepted articles for which immediate publication would seem important will be inserted in the next issue of the journal to go to press if the author is willing to pay the manufacturing cost. The insertion of such articles will not affect the publication of other manuscripts awaiting their turn, since the inserted articles will constitute additional pages to the volume without additional cost to the subscriber.

Manuscripts and Books for Review should be sent to Dr. L. W. Hackett, School of Public Health, University of California, Berkeley 4, California.

Correspondence concerning business matters, including advertising, should be addressed to The Williams & Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Subscription price: $10.00 per volume, United States; $10.50 Canada; $11.25 elsewhere.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be refunded to the subscriber.

Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Change of Address: Publisher must be notified 60 days in advance. Journals undeliverable because of incorrect address will be destroyed. Duplicates can be obtained (if available) from the publisher at the regular price of single issues.

THE WILLIAMS & WILKINS COMPANY
BALTIMORE-2, MARYLAND, U.S.A.

NATIONAL FAT STAINS

Techniques and solutions for the demonstration of fat in tissue have advanced markedly since the first use of oil soluble dyes for this purpose in 1896.

Using alcohol as the solvent for the dye, early workers in many instances dissolved out much of the fat present. Subsequently, solutions based on equal parts of 70% alcohol and acetone were used, and more recently solutions 50% to 60% isopropanol[1] and ethylene glycol or propylene glycol[2] have found favor.

Advances in solutions have been paralleled by better techniques as well as the availability of a wider selection of fat stains. National has steadily expanded its line of Biological Stains and Indicators to include the principal stains in current use for demonstrating fat in tissue. For your convenience, we list their Catalog Numbers:

#673 – Sudan II	#629*– Sudan Black B
#635*– Sudan III	#689 – Oil Red O
#542*– Sudan IV	#599 – Oil Blue N
#707 – Sudan Brown	#686*– Nile Blue A

*Certified by the Biological Stain Commission

1. Lillie, R. D., Histopathologic Technic 1948, pp. 157-160.
2. Chiffelle and Putt, Stain Technology, 26:51, 1951.

PHARMACEUTICAL LABORATORIES
NATIONAL ANILINE DIVISION
ALLIED CHEMICAL & DYE CORPORATION
40 RECTOR STREET, NEW YORK 6, N.Y.

(In writing to advertisers, please mention the journal—it helps.)

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

Papers should be written in *English*.

Manuscripts should be typewritten using *double or triple spacing* on *one side* only of 8½ x 11 inch paper, if available, and submitted in duplicate.

Tables should be typed on separate sheets and be serially numbered.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

Snyder, T., and Meleney, H., 1941. The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21:** 63–73.

Citation in the text should be by author and date.

Proprietary names of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U.S. Pharmacopoeia, New and Nonofficial Remedies of the A.M.A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

All papers should end with a brief summary.

Estimating costs of reprints. Where the cost of reprints must be known well in advance of publication in order to secure prior approval for their purchase, the Editor will be glad to furnish an estimate of the number of pages which the article will occupy in the Journal.

(In writing to advertisers, please mention the journal—it helps.)

SMITH SPRAYERS

"Choice for Quality the World Over."

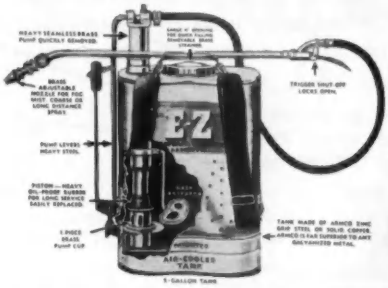
For Mosquito & Malaria Control

For Residual House Spraying or Larviciding Operations. Herculoy Silicon Bronze Sprayers are available in 4 gallon and smaller sizes and Armco Zinc-grip steel sprayers are also available in smaller sizes.

SEND FOR CATALOG

On our complete line of hand, continuous, compressed air, knapsack, bucket, wheelbarrow and barrel sprayers. Also hand and crank powder insecticide dusters. We are the originators of sprayers and our products are of outstanding quality in engineering, modern design and performance.

SMITH E-Z KNAPSACK SPRAYER



Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Tank solid copper or Armco zinc-grip steel. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight—20 lbs.

SMITH D.D.T. SPRAYER

The SMITH DDT Sprayer consists of a 4 gallon heavy welded galvanized corrugated steel tank, tested for hard service; equipped with brass air pump and brass air-check valve; adjustable carrying strap with snap ends, air-pressure gauge, 7-foot length of oil resisting hose; automatic shut-off control; 2-foot extension rod and all brass precision-built adjustable or fixed type nozzles. A custom built, superior sprayer. Heavy duty industrial use.

One in case.
Shipping weight—13 lbs.

SMITH No. 16D DUST KING (quart-size) DUSTER

Unexcelled in performance. Has floating plunger; does not wear out; very easy to operate. Superior in design and construction. Approved by Experiment and Extension Services.

SMITH No. 44 GIANT DUSTER

The Finest Made—Universally Approved

Unexcelled for mosquito and malaria control—when dusting is being done. Duster is precision built, well balanced, very light to carry and easy to operate. Superior in quality and performance.

One in case.
Shipping weight—12 lbs.

D. B. SMITH & COMPANY, 432 MAIN ST., UTICA 2, NEW YORK, U. S. A.

ESTABLISHED 1888

FIRST . . . *and best!*

TODD INSECTICIDAL FOG APPLICATOR

TIFA

Successfully Used for over 10 Years in Community Fly and Mosquito Programs

Tifa was first in the field of fog applicators. Tests have proved it best in the field for space control around the community . . . on the dump, over pools, in suburbs, everywhere insects swarm!

This amazing machine discharges newly-developed insecticides as well as the older types in a true, clean fog which spreads quickly and permeates the deepest crevices. This ability means a better insect control program for your community. Put Tifa to work for you!

EFFICIENCY FEATURES

Uses insecticides in solutions, emulsions or suspensions.
Produces penetrating fog, dry or wet as you wish.
Droplet Control ranges from ½ to 200 Microns.
Carrying Underwriters' Listing Seal

Write for Illustrated Folder with Full Details and Official Comments.

PRODUCTS DIVISION
TODD SHIPYARDS CORPORATION

Headquarters:
Columbia & Halleck Streets, Brooklyn 31, N. Y.
Plant:
Green's Bayou, Houston 15, Texas

THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

APPLICATION FOR MEMBERSHIP

To the Membership Committee:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

(TYPE OR PRINT)

Name..... (*Last*) (*First*) (*Middle*)

Mailing Address: Street..... City.....

State..... Country.....

Date of Birth..... Place.....

Special Field of Interest.....

Education: Institution (Graduate and Postgraduate) Degree Date

.....

.....

.....

Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

*Proposed by:

(1)..... .....

(2)..... Signature of Applicant

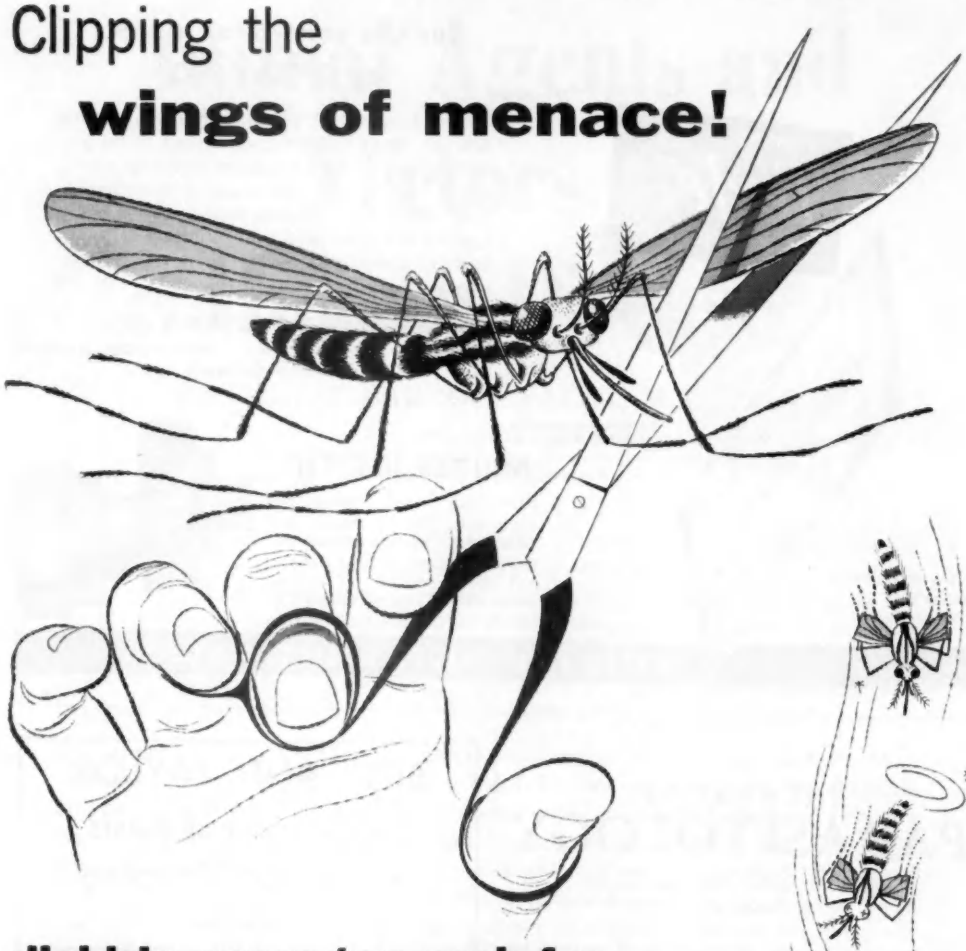
*Sponsorship by two active members is obligatory.

Return this application to Dr. John E. Larsh, Jr., Secretary-Treasurer, School of Public Health, University of North Carolina, Chapel Hill, North Carolina, U. S. A.

(*Check for $8.00 (U. S. banks requested) must accompany this application.*)

(In writing to advertisers, please mention the journal—it helps.)

Clipping the wings of menace!



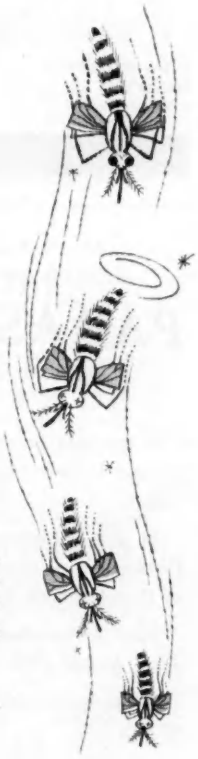
dieldrin earns top rank for mosquito control 'round the world

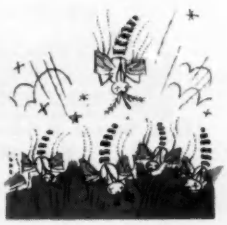
Dieldrin's power to combat insect vectors of disease is well recognized. Pest control operators and government mosquito abatement crews, by using dieldrin, can deliver vast areas from the ravages of contagious and epidemic diseases.

Dieldrin halts larval development . . . one application per season generally proving sufficient. It controls adult mosquitos of all species, and is effective against flies, ticks, chiggers and fleas.

• • •

Dieldrin formulations are readily available from insecticide distributors.





dieldrin

SHELL CHEMICAL CORPORATION

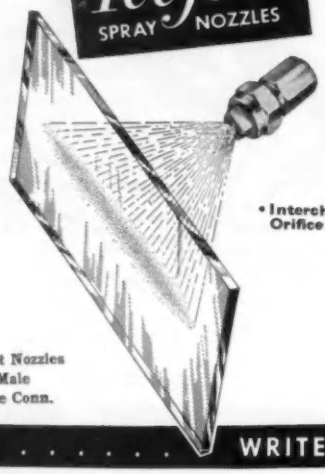
AGRICULTURAL CHEMICALS DIVISION · P. O. BOX 1617, DENVER 1, COLORADO

Atlanta · Houston · New York · San Francisco · St. Louis · Jackson, Miss.

(In writing to advertisers, please mention the journal—it helps.)

for the control of insects by SPRAYING

SPRAYING SYSTEMS
TeeJet
SPRAY NOZZLES



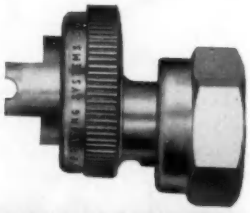
Proper atomization plus uniform distribution are the spray characteristics that make precision-built TEEJET nozzles ideal for spraying of insecticides. Full range of interchangeable orifice tips provide capacities for every type of chemical and every method of application. Complete nozzle includes built-in strainer with monel metal screen.

SPRAYING SYSTEMS CO.
3234 RANDOLPH STREET • BELLWOOD, ILLINOIS
(Suburb of Chicago)

• Interchangeable Orifice Tips

MulTEEJET TIP

For use with portable sprayers. Turret head indexes to four positions to give selection of four different spray patterns. Can be used with Trigger TEEJET for handy shut-off control.



• TeeJet Nozzles Built in Male or Female Conn.

WRITE FOR TEEJET BULLETIN NO. 58

JOURNAL OF
PARASITOLOGY

Official Organ of American Society of Parasitologists

A medium for the publication of papers on Animal Parasites (Protozoa, Helminthes, Arthropoda).

It also publishes the program and abstracts for the annual meeting of the American Society of Parasitologists in a Supplement.

Regular numbers appear in February, April, June, August, October and December.

Subscription price, **$7.50 the year** (Foreign, $7.90).

GEORGE R. LA RUE
Chairman, Editorial Committee

JOURNAL OF PARASITOLOGY
U. S. Bureau of Animal Industry, Zool. Div.,
Beltsville, Maryland

BEST AND TAYLOR
Physiological Basis of Medical Practice

Sixth Edition

The *U. S. Armed Forces Medical Journal* said of an earlier edition of Best and Taylor: "This book should be in the library of every physician regardless of his specialty, for the practice of medicine is founded upon a thorough knowledge of physiology." The *New York State Journal of Medicine* calls Best and Taylor a "must book for every doctor's library."

The thoroughly revised sixth edition fully merits the praise given earlier editions. If your edition is earlier than the fifth, it's time for a new one.

1370 pages over 600 figs. $12.00

The Williams & Wilkins Co.
Mt. Royal and Guilford Aves.
Baltimore 2, Md.

Animal Agents and Vectors of Human Disease

By ERNEST CARROLL FAUST, M.A., Ph.D.

The William Vincent Professor of Tropical Diseases and Hygiene and Head, Division of Parasitology, Department of Tropical Medicine and Public Health, The Tulane University of Louisiana, New Orleans

PARASITISM constitutes one of the major phenomena of the living world, and therefore one of its most interesting and challenging subjects. A highly important phase of parasitology is the broad area involving *animal agents and vectors, and their relation to disease.* Dr. Faust, internationally known for his work in this field, presents a full discussion of those parasites which produce human disease. The etiology, epidemiology, pathogenesis, symptomatology, diagnosis, treatment, and control of this group of infections are considered in detail.

The presentation is intentionally not exhaustive, yet the volume is much more than an outline syllabus. In extensive, helpful tables in the introductory chapters, Dr. Faust tells where each parasite under discussion is found, its usual infective stage, portal of entry, primary and secondary sites, usual reservoir hosts (monkeys, dogs, rats, etc.), and other obligate hosts and vectors. Pathologic effects and clinical manifestations produced by animal agents of disease, how to diagnose them, the most satisfactory therapeutic procedures and methods of control, etc., are presented in a similar manner. Subsequent chapters give detailed information on each agent or vector as it is considered individually.

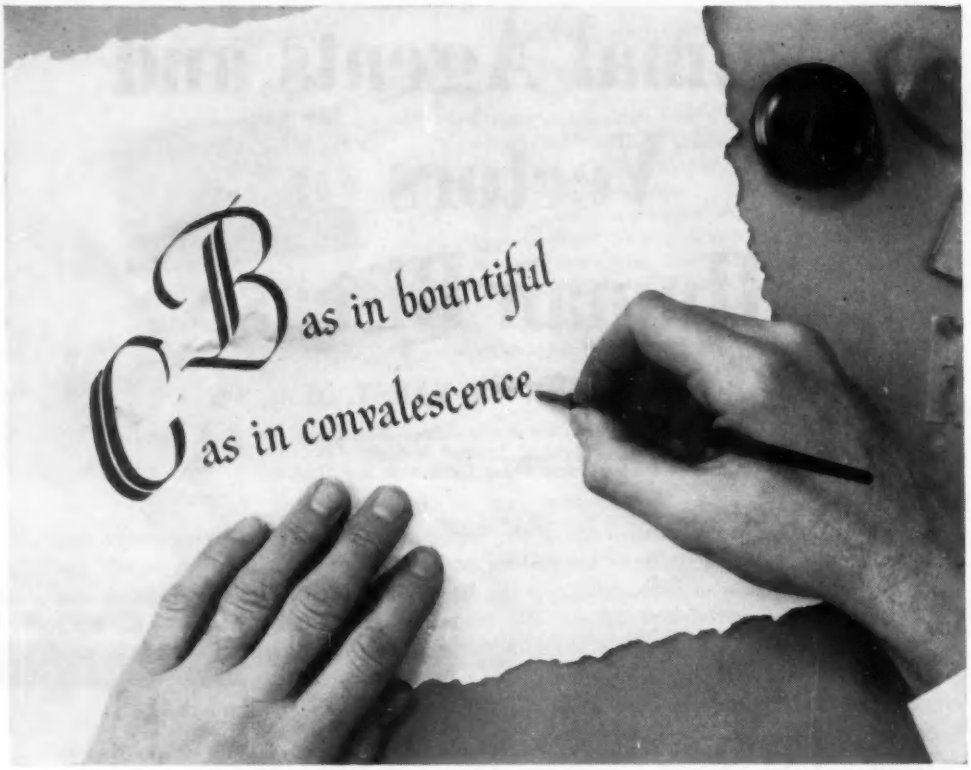
Morphologic features of the organisms involved and the clinical aspects of the diseases which they produce, are elucidated by numerous helpful illustrations, many of which were made for this book. Ample references conclude each chapter. This important and timely work is designed for use by zoologists, epidemiologists, pathologists, practicing physicians, laboratory diagnosticians and public health workers.

660 Pages. 216 Illustrations and 9 Plates, 1 in Color. 12 Tables. $9.75.

(Published January, 1955)

LEA & FEBIGER WASHINGTON SQUARE PHILADELPHIA 6 PA.

(In writing to advertisers, please mention the journal—it helps.)



'Becotin with Vitamin C'

(VITAMIN B COMPLEX WITH VITAMIN C, LILLY)

a distinguished member of the Lilly family of vitamins

. . . To enhance convalescence in major surgery, severe burns, and febrile, gastro-intestinal, and wasting diseases; or whenever stress situations threaten to cause vitamin depletion. Often a helpful supplement in antibiotic therapy.

'Becotin with Vitamin C' combines in one capsule therapeutic amounts of vital synthetic vitamins of the B complex plus ascorbic acid and all the vitamins naturally occurring in desiccated liver and stomach tissue.

In bottles of 100 and 500.

Lilly

QUALITY / RESEARCH / INTEGRITY

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U.S.A.

(In writing to advertisers, please mention the journal—it helps.)

INTRODUCTION TO THE SCHISTOSOMIASIS SYMPOSIUM[1]

DONALD B. McMULLEN

Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.

It was supposed that the Program Committee had an ulterior motive in suggesting a symposium on schistosomiasis. With this in mind the panel's approach has been directed primarily toward the control of the disease. Since I recently had the opportunity to express my views on this problem (McMullen, in press), the introductory remarks will be brief so that the members of the panel will have as much time as possible.

Schistosomiasis, like yellow fever, typhus and malaria, is an extremely complicated bio-economic problem. At present, however, its control has not reached the same degree of proficiency. To attain this may require the efforts of experts in the fields of agriculture, biology, chemistry, ecology, education, engineering, epidemiology, malacology, medicine and public health. Statistically our best chance of success is to have representatives from each of these fields combining their efforts to solve this problem. There is evidence that this phase is beginning but we can not yet be certain which approach warrants the greatest attention.

It has been stated that schistosomiasis is now in the stage that is analogous to the quinine-Paris green phase of malaria control (Meleney, 1954). That our therapeutic drugs are inadequate and our molluscicides impractical, except under limited conditions, is usually accepted by realists. The indications are that we have a long way to go, and that it will take a great deal of exploration to find the best combination of methods. It is encouraging to recall that investigations of other diseases indicate that some adventurous or plodding endeavor eventually shows the way over or around the worst obstacles. Even when this point has been reached new ones may appear, analogous to jungle yellow fever, resistant strains of vectors, etc., in the other diseases; but in general the path will be more clearly marked than it is now.

At present there are investigations in progress that may materially assist in solving this problem. Attempts are being made to gain a perspective of the parasites involved, to study the closely related species, to consider their physiology, and to examine the effect on their hosts. There is evidence of a concentrated effort on the study of the bionomics and ecology of the snail vectors. There are indications that further studies on molluscicides would be rewarding. For example, one of the compounds tested in our laboratory during the past year was reported to be lethal for fish at concentrations of 0.007 p.p.m., but mice were said to tolerate 250 mgm/kilo. Unfortunately our tests showed that the compound was only mildly toxic to snails, but the mere knowledge that such a compound exists is encouraging. One may be found that will reverse the snail-

[1] Presented at the joint meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists, Memphis, Tenn., 3–6 November, 1954.

fish activity and still be relatively innocuous to mammals. Because of its unusual properties this compound was also tested as a therapeutic drug but the worms were unimpressed.

The members of this panel will report work done on the respective topics listed, point out gaps in our knowledge, and attempt to indicate directions for future work. Each speaker has been selected because of his investigations on the phase of the subject that he will discuss. You have already identified them by their work in Egypt, Brazil, Japan, Puerto Rico, and in the clinic. They do not represent all the scientific fields mentioned above but they are well qualified to discuss what appears at present to be some of the most active phases of research on schistosomiasis.

REFERENCES

McMULLEN, DONALD B., in press. Some of the problems in schistosomiasis control, *Proc. Eighth Pacific Science Congress* (1953).

MELENEY, HENRY E., 1954. Problems in the control of schistosomiasis, *Amer. Jour. Trop. Med. & Hyg.* **3**: 209–218.

BIOLOGY OF THE SCHISTOSOME COMPLEXES[1]

ROBERT E. KUNTZ

Parasitology Department, Naval Medical School, National Naval Medical Center Bethesda, Maryland

For years schistosomiasis was considered as a disease of man and animals in scattered but somewhat localized geographical regions of the world. In the past decade however it has been recognized as one of the most important parasitic diseases. Various interests and circumstances through the years have more or less governed the pace and the trends of laboratory and field work relating to the biology of the parasites responsible for the schistosomiases in different countries. An intermingling of races and types of people, and the movement of large numbers of troops from one geographical area to another during the war aroused concern, and renewed fear, that military and related personnel might contract schistosomiasis in foreign lands and possibly introduce the disease into new countries. Although there had been a steady interest and a constant accumulation of knowledge on many phases of schistosomiasis, these new circumstances or factors stimulated studies on a much greater scale than before.

It is now realized that the status of schistosome problems may change rather rapidly and that each geographical area is characterized by species of schistosomes which may affect man or lower animals, or both. One or two species may be true assaulters of man while others may be schistosomes of birds and lower mammals, attacking man only superficially. In the latter case varying degrees of dermatitis may result.

A general study of the biology of the schistosomes may be approached from the standpoint of parasite-definitive-host and parasite-intermediate-host complexes in different geographical regions. If we are to consider the schistosomes of greater concern to man and animals on a geographical basis, they fall into the following regional categories: Africa and the Middle East; Portugal; India; the Orient including China, Japan, the Philippine Islands and Formosa; and the Americas. This breakdown is one of convenience for discussion rather than an attempt to segregate the schistosomes by species or according to accepted faunistic divisions.

AFRICA AND THE MIDDLE EAST

At the present time, the principal schistosomes of man and lower mammals in Africa fall into two general categories or complexes; those of the *Schistosoma*

[1] The opinions and assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The author wishes to express his appreciation to Dr. H. F. Hsü, Department of Hygiene and Preventive Medicine, State University of Iowa, Iowa City, Iowa, who has provided unpublished manuscripts describing the work by him and his colleagues in Formosa and to Dr. G. R. La Rue, Bureau of Animal Industry, USDA, Beltsville, Maryland who has kindly read the manuscript.

haematobium-type with terminally-spined eggs; and those of the *S. mansoni*-type with laterally-spined eggs.

After the discovery of *Schistosoma* by Bilharz in Cairo, there followed a period of debate by well known parasitologists on the relationship of schistosome parasites possessing eggs with terminal or with lateral spines. For a few years after the validity of *S. mansoni* and *S. haematobium* had been established, it was assumed that man, at least, was infected with only two species of *Schistosoma* in Africa. Doubt was soon cast on this belief however as Cawston (1921 a) in South Africa found in the urine of man, eggs resembling those of *S. bovis*, a parasite described much earlier from cattle in Egypt by Sonsino. Adults of the parasite were obtained by experimental infection of laboratory animals with cercariae obtained from *Physopsis*. Thus began a series of investigations and reports describing the parasitism of man and animals by *haematobium*-like schistosomes producing various shapes of terminally-spined eggs. Veglia and LeRoux (1929), also working in South Africa, announced the finding of a new schistosome, *S. matthei*, from sheep. This discovery was followed by reports of the same parasite occurring in cattle, baboons and also in man in Southern Rhodesia (Blackie, 1932). MacHattie and Chadwick (1932) found *S. bovis* in horses, donkeys and mules in Iraq. After a detailed consideration of the morphology and egg types of a large series of parasites they expressed the belief that *S. bovis* as seen in Iraq was identical with the *S. matthei* of Veglia and LeRoux in South Africa. They noted the variability in the shape of the eggs produced by their schistosomes and placed emphasis on the fact that some of the eggs of *S. bovis* were indistinguishable from those of *S. haematobium* in man.

Fisher in 1934 described another parasite of the *S. haematobium* group, *S. intercalatum*, from man in the Stanleyville area of the Belgian Congo. This parasite was indistinguishable from *S. bovis* and *S. haematobium* except for differences in the measurement and shape of the eggs. Fisher was cognizant of the extremes in variation of the eggs but felt certain that those of *S. intercalatum* could not be confused in shape with those of *S. haematobium* nor in their measurements with those characteristic of *S. matthei* or *S. bovis*. After measuring a large number of eggs, he employed length frequency curves to support the contention that he was working with a definite species intermediate between *S. haematobium* and *S. bovis*. Of considerable significance was Fisher's observations that *S. intercalatum* in the Stanleyville area was responsible for intestinal schistosomiasis in contrast to vesical involvement resulting from infection by *S. haematobium* in other areas of the Congo.

Although some consideration was given to certain clinical aspects of schistosomiasis and to the location of the parasites in the host, the measurements and characteristic shape of eggs steadily gained a more important role in schistosome recognition. Pros and cons were expressed regarding the advisibility of accepting egg characters alone as a means for separating related species. This criterion to some became even more objectionable as the same schistosomes were reported in both lower mammals and man. Lane (1936) after reviewing the accrued data of various investigators on the taxonomic and biological status of the

schistosome problem declared that *S. matthei* was not a separate species. In the following year, van den Berghe (1937) expressed his belief that the lines of demarcation between *S. haematobium*, *S. bovis* and *S. matthei* are not sharp and suggested that *S. matthei* be called *S. bovis* var. *matthei* and *S. intercalatum*, *S. haematobium* var. *intercalatum*.

Alves (1949) was of the opinion that eggs could be distinguished more accurately if consideration were given to a third dimension, i.e. breadth of the egg at an arbitrary distance of 50 μ from the non-spiked end which would tend to emphasize the spindle shape of the egg as in the case of *S. bovis*. By employing the data from measurement of 500 eggs of *S. bovis*, *S. matthei* and *S. haematobium* he was able to differentiate between the three types of eggs by statistical analysis. He conservatively considered the use of his formula as an approach to, rather than as an absolute criterion for species differentiation, especially of the eggs of *S. matthei* and *S. bovis*. However, his observations supported his contention and others that *S. matthei* could possibly infect man.

Data on the controversial subject of infection of man by schistosomes of lower mammals and vice versa have continued to accumulate. Thus Raper (1951) reported that he was personally infected with *S. bovis*, and that eggs observed in feces satisfied the criteria for this parasite as laid down by Alves. More recently de Meillon, *et al.* (1953) in South Africa have concluded that human beings are not readily susceptible to infection by *S. bovis* since only one case was detected from the urines of 910 children in an area where many *Physopsis* were known to be heavily infected.

Schwetz (1951a) provided more realistic means for evaluation in the differentiation of these species of schistosomes of questionable status since he had an opportunity to make comparative studies of adult morphology as well as egg characteristics of *S. bovis*, *S. haematobium*, *S. matthei* and *S. intercalatum*. Furthermore his observations and collections of material in the field were greatly enhanced by subsequent careful and more detailed biological investigation in the laboratory. He showed that all four species were similar morphologically, all were transmitted by the same snail host, *Physopsis* (in the Belgian Congo) and all four produced terminally-spined eggs. However, there were differences in the egg shapes. Eggs of *S. haematobium* differed from those of *S. intercalatum* and *S. matthei* and the latter two differ in shape from those of *S. bovis*. Schwetz stated that *S. bovis* was the cause of disease in animals, whereas *S. haematobium* and *S. intercalatum* were pathogenic to man, the former primarily involved in vesical schistosomiasis in the Belgian Congo and *S. intercalatum* in the case of the intestinal form of the disease. If the latter relationship of parasites to position in the host body is employed as a criterion for differentiation of schistosomes in the Congo, it might be well worth the effort to investigate the intestinal type of so-called *S. haematobium* infection in Egypt, the Middle East and other parts of Africa. Careful studies may reveal unexpected inter-specific *haematobium*-like complexes in other parts of the continent.

A scheme in which egg measurements or length-breadth ratios were employed to indicate species or subspecies relationships for parasites of the *haematobium*-

complex of Africa has been proposed by Amberson and Schwarz (1953). In their plan, one not readily accepted by all investigators in schistosomiasis work, all species of the *haematobium*-complex appear as subspecies of *S. haematobium*. The inadvisability of giving too much weight to shape as a criterion for recognition of schistosome eggs was emphasized by Vercruysse (1953) who described the predominant polymorphism of *S. bovis* eggs.

The *haematobium*-group of Africa may be represented further by *S. curassoni* and *S. spindalis*. The status of both species is questionable. Brumpt (1931) described the former from the portal system of cattle, stating that this form was closely related to *S. matthei*. *Schistosoma spindalis* is normally a parasite of cattle in India and Malaya but the eggs of this parasite were reported from the urine of man by Cawston (1925) and from both feces and urine of man in South Africa by Porter (1926). Although based upon fragmentary evidence, the latter designated the parasite as *S. spindalis* var. *africana*. More recently LeRoux (1948), considering the schistosomes of cattle and wild animals of South Africa, exhibited a specimen which he described as closely related to *S. spindalis* but distinct from the schistosomes of the same name from India. Fairley (1951) asserted that there is no evidence that *S. spindalis* has ever developed in man.

The second category of schistosomes in Africa for years was represented by a single supposedly stable parasite, *S. mansoni*. The status of this group was changed in 1951 when Schwetz and Stijns rediscovered the almost forgotten *S. rodhaini*, described 20 years earlier by Brumpt (1931). *S. rodhaini* is a parasite of wild rodents, differing from *S. mansoni* in certain of its biological characteristics and in the shape of its eggs. Typical eggs may be differentiated from all other schistosome eggs in having a subterminal spine and a narrow opposite extremity which is turned in the opposite direction from the spine. *S. rodhaini* matures much earlier than *S. mansoni* and the ensuing infection is much more acute, resulting in a greater number of deaths among experimental hosts. In certain areas, this schistosome commonly infects a number of rodents represented by *Lophuromys*, *Praomys*, *Pelomys* and *Mastomys* (Stijns, 1952). *Crocidura*, an insectivore, in the same area was negative for *S. rodhaini* but possessed eggs of *S. mansoni* in the intestine. Stijns (l.c.), working in conjunction with Schwetz, examined native laborers engaged in clearance of canals containing snails infected with *S. rodhaini*. None were infected with *S. rodhaini* but all were heavily infected with *S. mansoni*. The author concluded that *S. rodhaini* in nature possibly is found parasitic only in rodents.

As a result of his extended studies and surveys Schwetz (1953) discovered and described still another *mansoni*-like parasite in the Katanga Province of the Belgian Congo. This was later designated as *S. mansoni* var. *rodentorum*. Both the latter parasite and *S. rodhaini* were first obtained by experimental infections of mice; then later were found in naturally infected rodents. Schwetz placed both in the *S. mansoni* group by virtue of their anatomy as well as by the fact that they had the same intermediate host. The infections were light and chronic and were well tolerated by the host. Incidence of the infection was lower than

for *S. rodhaini* and its eggs were similar to the lateral-spined eggs of the conventional *S. mansoni*.

Leiper (1918), in his first attempts at life history work in Egypt, found it necessary to expose various small mammals as well as primates to the cercariae of the schistosomes to provide adults for study purposes. Numerous references to the ability of the schistosomes of man and livestock to infect small mammals have appeared since that time. Only in recent years however have susceptibility studies been employed as a means to learn more of the fundamental biology of the schistosomes. In this respect, the schistosomes of the *haematobium*-complex have received much less attention than *S. mansoni*. Reports of low grade infection or failure of *S. haematobium* to establish itself in small mammals in previous incidental researches have discouraged the use of this parasite for experimental schistosomiasis.

Susceptibility data above all have demonstrated that the schistosomes of Africa are not nearly as host specific as once supposed. Becker (1916) and Cawston (1921b) seem to have been among the first to demonstrate that *S. haematobium* could be obtained by experimentally infecting guinea pigs. Ingram (1924) infected wild rats with *S. haematobium* in West Africa. Brumpt (1928) included guinea pigs, white mice, white rats, hedgehogs and monkeys in a list of animals reported as having been infected with *S. haematobium*. Later Brumpt and Chevallier (1931) studied experimental schistosomiasis in white mice resulting from infection with *S. haematobium*, *S. mansoni* and *S. bovis*. In Southern Rhodesia Blackie (1932) successfully infected rabbits but was unable to infect guinea pigs with *S. haematobium*. Gillet (1949), on the other hand, was unable to infect a series of 33 guinea pigs with the cercariae of *S. haematobium* from *Physopsis* in the Belgian Congo but did pass the infection into white mice. In the following year, Abdel Azim and Cowper (1950) were unsuccessful in infecting white mice with the Egyptian strain of this parasite. Reports covering such a range of results are somewhat disillusioning, especially in view of Alves (1948) statement that he found it impossible to infect any of the small laboratory animals with *S. haematobium*.

Records pertaining to the susceptibility of small animals to other schistosomes of the *haematobium*-complex are less numerous. Cawston (1921b) recovered *S. bovis* from guinea pigs and Dowdeswell (1938) obtained the same parasite from laboratory mice. Porter (1938) reared *S. bovis* to maturity in rabbits, guinea pigs and rats. Adults of *S. intercalatum* were raised in white mice by Fisher (1934) and Schwetz (1951b). The latter also obtained *S. bovis* from mice in Uganda. According to Alves (1948), *S. matthei* was capable of infecting any of the commonly used laboratory animals. He obtained study materials from guinea pigs, hamsters and mice. Blackie (1932), also working in Southern Rhodesia, reared *S. matthei* in guinea pigs but apparently found them sexually immature. Abdel Azim and Cowper (1950) successfully infected Egyptian desert gerbils (*Gerbillus*) with *S. matthei* cercariae from snails imported from Southern Rhodesia. A number of primates, listed by Kagan (1953), have been employed for experimental schistosomiasis.

Susceptibility studies by Moore and Meleney (1954) in the United States and by Kuntz and Malakatis (1955a) in Egypt, evaluating the use of laboratory animals as hosts for *S. haematobium* of Egyptian origin, certainly do not agree with LeRoux's (1948) startling statement that "there is no proof that *S. haematobium* has ever been raised to full maturity in mice and guinea pigs." These investigations as well as an earlier one by Meleney *et al.* (1953) have demonstrated that white mice and hamsters can be used satisfactorily as experimental hosts with passage of eggs in feces. Although not consistent, infections in the hamster gave rise to bladder lesions. Albino rats and guinea pigs were poor hosts and rabbits were refractory to infection in both studies. Kuntz and Malakatis (l.c.) further demonstrated that carnivores in general were poor hosts. They were unable to infect dogs and found only negligible numbers of poorly developed schistosomes in cats. Baboons (*Papio*) and monkeys (*Cercopithecus*) were easily infected with *S. haematobium* but the yields of worms and production of eggs in feces and urine were variable. Infection was established but only a few *S. haematobium* were found in goats exposed to several thousands of cercariae.

Further studies by Kuntz and Malakatis (unpublished data) have demonstrated that all of the common rodents of lower Egypt were susceptible to infection by *S. haematobium*. The list included several desert rodents (*Gerbillus*, *Jaculus* and *Meriones*) and domestic murines (*Mus muculus* and *Rattus rattus*) as well as the Nile rat *Arvicanthis niloticus*. The bandicoot (*Nesokia*), the spiny-back mouse (*Acomys*) and hedgehogs (*Hemiechinus*) were also infected. The Nile rat was the best host because of its ability to maintain numerous worms which passed viable eggs for approximately three years.

Investigations pertaining to the infection of various hosts by *S. mansoni* in Africa are less numerous. Recent studies (Kuntz, *et al.*, 1954) demonstrated that the Egyptian "strain" of *S. mansoni*, like that of the western hemisphere, was capable of infecting most of the commonly used laboratory animals. Emphasis on the white mouse, a proven satisfactory host, showed that the Egyptian strain produced greater quantities of eggs than the Puerto Rican strain. Consequently, the Egyptian *S. mansoni* was responsible for more severe damage and in some cases earlier death to the experimental hosts. Although the dog has been regarded as an incompatible host for *S. mansoni* in the United States, the study in Egypt revealed that occasionally an individual dog may show partial compatibility. One of eleven dogs, five weeks after exposure to several thousand cercariae, possessed approximately 300 small immature schistosomes in the liver. During the course of the same study, two goats were lightly infected with the Egyptian *S. mansoni*.

Susceptibility studies in different laboratories working with wild mammals have provided a long list of hosts which can be infected by *S. mansoni*. Buttner (1953) exposed 12 species of rodents to *S. mansoni* originating from Egypt, French Guinea and South America. All species of rodents, wild as well as domesticated, were successfully infected. Kuntz and Malakatis (1955b) exposed the same series of wild mammals to *S. mansoni* as employed in experimental studies with *S. haematobium*. All species were infected and *Arvicanthis* proved to be the

best host. Carnivores were poor hosts. Mongooses (*Herpestes*) were difficult to infect while weasels (*Mustela*) and wild foxes (*Vulpes*) produced no schistosomes. The hedgehog, an insectivore, was only a fair host. Its susceptibility was classed as intermediate to rodents and carnivores.

A search for wild mammals to determine their possible role as reservoirs in Egypt resulted in finding a single gerbil (*Gerbillus*) infected with *S. mansoni* (Kuntz, 1952). Later examinations (Kuntz and Wells, unpublished data) revealed a number of infected shrews (*Crocidura*) from the banks of irrigation canals near Cairo.

Announcement by Deramee *et al.* (1953) of the discovery of a focus of canine schistosomiasis in the Belgian Congo due to *S. rodhaini* was of considerable importance from the standpoint of susceptibility relationships. A number of dogs heavily infected with *S. rodhaini* and displaying obvious symptoms as well as passing eggs in the feces suggested a well established compatibility of parasite and host. Later attempts to infect cats and dogs as well as a series of rodents experimentally were all successful (Fain *et al.*, 1953). Such a disclosure introduces an unexpected factor in our present concept of the ability of schistosomes of the *mansoni*-complex to infect canines. Previously repeated attempts to infect laboratory dogs with *S. mansoni* of American and Egyptian origin usually met with failure. Similar attempts with *S. haematobium* (Kuntz and Malakatis, 1955a) were likewise negative. In *S. rodhaini*, we see the first indication of an African schistosome with unusual range of infectivity for definitive hosts, a feature so well known for *S. japonicum* in the Orient.

Experimental studies with *S. rodhaini* have provided additional basic information of extreme interest and importance. LeRoux (1954a) successfully hybridized *S. mansoni* and *S. rodhaini*. Eggs produced by the hybrids showed that the position of the spine and other characteristics were intermediate to those usually found in the eggs of the parents. Furthermore, it was observed that the eggs of the hybrids had a close resemblance to those of the recently described *S. mansoni* var. *rodentorum*.

Data gained from the various susceptibility studies of schistosomes of the *haematobium*- and the *mansoni*-complexes, plus the information provided by Schwetz's (1953, 1954a) finding of the rodent strains of *S. mansoni*, indicate the broad scope of possibilities in host-parasite relationship problems for the future. It also stimulates a certain amount of speculation regarding the basic relationships of the schistosomes of lower animals to those found in man. Schwetz has pointed out that *S. rodhaini* is related to *S. mansoni* just as *S. bovis* is related to *S. haematobium*. Naturally the question comes to mind "What was the original parasite-host combination?" Are these new morphological and biological "strains" and "forms" adaptations of human parasites to lower mammals or have modifications resulted from the schistosome passing from wild rodents to man? Although the distribution of his new forms is unknown and the overall significance of Schwetz's work cannot be evaluated at present, it opens new fields of investigation in the approach to the basic biology and the epidemiology of schistosomiasis.

It was believed at one time that a knowledge of the morphology of the cercaria offered a means for recognition and differentiation of the species of schistosomes. The study of cercariae demands careful handling, patience and very exacting observations; consequently there has been considerable disagreement on the morphology. Unfortunately, the disagreement has rendered this phase of study more of a detriment than an asset in an understanding of schistosome biology.

Special attention has been given to the excretory system and to the number and characteristics of the penetration glands as criteria for differentiation. An appraisal of the morphology of cercariae of the two major schistosome complexes of Africa based upon descriptions available in the literature indicates that in all probability, if examined after natural emergence from their snail hosts, all possess five pairs of flame cells, *i.e.*, four pairs in the body and one in the tail, and five pairs of penetration glands. Fain (1952) and his colleagues (Fain *et al.*, 1953) have recently described the morphology of *S. intercalatum* and *S. rodhaini* respectively from the Belgian Congo. Their descriptions agree in general with earlier accounts (Gordon *et al.*, 1934) of the morphology of *S. mansoni* and *S. haematobium* in West Africa and with the present author's observations of the latter two species in Egypt.

It seems likely that attention must be turned to other features of cercariae if diagnostic characteristics are to be found. In this respect, the presence of delicate hair-like processes originating from papillae on the surface of cercariae may offer a possible lead. These fine structures had been recognized previously in the cercariae of *S. mansoni* and *S. haematobium* (Gordon *et al.*, 1934) and in *S. japonicum* (Kuntz, 1950) but were given no serious consideration until Vercammen-Grandjean (1951) demonstrated their constancy in number and symmetrical arrangement in the cercariae of *S. mansoni*. In a note appended to his paper, it was stated that these structures had also been observed in the cercariae of *S. bovis*. Fain and his co-workers (l.c.) have also called attention to the same processes. Certainly these structures, although demonstrable only in the living cercariae, deserve further study. Possibly a method can be developed by which accurate observations of these features may allow a taxonomic separation of schistosome species based upon a system of "chaetotaxy". Relative measurements of the parts of cercariae could be a very helpful adjunct to cercarial identification if a standard method for fixation and measurement were followed by all engaged in this type of work.

Parasite-snail as well as parasite-definitive-host relationships play a critical role in our understanding of the distribution, life cycle and the biology of the schistosomes. Among the mammalian schistosomes, the former association is more exacting than the latter, as indicated by susceptibility studies in which any one species has been shown capable of infecting numerous definitive hosts. As a rule, the same schistosome species is capable of infecting one or at most, several species of molluscs. Recognition of at least two mammalian schistosome complexes in Africa indicates the likely significance of parasite-snail problems or relationships. However, a discussion of the taxonomy of snail hosts, known, suspected or wrongly incriminated as transmitters of African schistosomiasis is beyond the scope of the present paper.

The first report of the Expert Committee on Bilharziasis (1953) of the World Health Organization expressed the dire need for additional studies on snail vectors to determine more accurately their transmitting potentialities, biology, ecology and possibility of adaptation of local strains of *Schistosoma* in any area to other species of snails. A plea for accurate and thorough studies in snail biology was particularly directed toward Africa. In an absence of an acceptable plan for snail taxonomy, it is difficult if not impossible to approach certain of the basic biological problems pertaining to schistosomiasis. The need for clarification of snail taxonomy in Africa was re-emphasized in a second report by a special study group of WHO (1954) and they presented a provisional list of schistosome vectors for Africa with general geographical distribution as recognized at the present time. They also gave specific techniques and the proper methods for preservation to be employed in collection of snails intended for taxonomic studies. It is hoped that the combined efforts and unified studies by a qualified group appointed for the task will clarify the present state of chaos in the field of snail taxonomy in Africa.

Amberson and Schwarz (1953) made a proposal for the taxonomy of the schistosomes and their vectors for the whole of Africa. This treatise contained sweeping statements, simplification in snail groupings which are not in agreement with the majority of earlier works, and conclusions regarding snail taxonomy and schistosome-snail relationships that are not accepted by many of those currently engaged in schistosome research. Possibly their contribution will provoke equally stimulating reports from other quarters. Schwetz (1954b) criticized the present status of snail classification for Africa and especially that concerned with the planorbids of the Belgian Congo. He gave vivid examples to illustrate the inconsistency and shortcomings of methods or characteristics employed for the differentiation of molluscs by different investigators. Surely it will be agreed that the schistosomes and the vectors which transmit them should be studied carefully as they occur in the field. In snail taxonomy too much reliance should not be placed upon dead shells in museums tagged with inaccurate or inadequate data. It is doubtful however, as once intimated by Schwetz, that we can disregard the International Code of Zoological Nomenclature in naming the molluscan hosts.

In Egypt, parts of North Africa and the Middle East, *S. mansoni* and *S. haematobium* are transmitted by *Biomphalaria boissyi* and *Bulinus truncatus* respectively. *Biomphalaria* (*Planorbis*) *pfeifferi* with its numerous synonyms seems to be the principal vector of *S. mansoni* in Central and South Africa. The exact distribution of this species and proper designations of subspecies are yet to be worked out. It has been generally stated that *Physopsis africana* and *P. globosa* and their subspecies are the intermediate hosts for *S. haematobium* south of the Sahara. The list of snails suspected or proven as vectors continues to grow. Just recently, Schwetz (1951b) and LeRoux (1954a) demonstrated *Physopsis nasuta* in Uganda and *Bulinus coulboisi* for the Belgian Congo respectively to be vectors of *S. haematobium* and Gaud (quoted by Cowper, 1953a) stated that *Bulinus* (*Isidora*) *madagascariensis* was the established vector in Madagascar. Cowper's (1953a) work in Mauritius gave *Bulinus* (*Pyrgophysa*) *forskali* as the

presumptive snail host for *S. haematobium* on that island although there was conflicting evidence on the grounds of epidemiology.

In Africa as elsewhere, the miracidia of schistosomes of one area have been exposed to known and suspected snail transmitters from other parts of the continent or to snails of questionable status in the same area. The number of instances in which such data have been presented to help explain geographical relationships of snails and schistosomes or to incriminate non-vectors are too numerous to cite. It seems that far too much emphasis has been given to the apparent attractiveness of snails for miracidia as a criterion in judging schistosome-carrying potentialities. However, the attention given to *Bulinus* (*Pyrgophysa*) *forskali* illustrates well the concern that may be elicited in field and laboratory studies for a species of snail suspected as a possible carrier of *S. haematobium*.

The suggestion that *B.*(*P.*) *forskali* might be of concern in schistosomiasis dates to the work of Leiper (1915) who after attempts to infect various molluscs stated that this snail showed attraction for schistosome miracidia. Apparently on the basis of this suggestion, investigators on numerous occasions have referred to *B.*(*P.*) *forskali* as the probable vector when no other snail could positively be incriminated. Further circumstantial evidence, followed by inquiries into the experimental as well as the practical aspects of the problem have revealed several points of interest. Adams (1934, 1935), searching for the snail host of *S. haematobium* on Mauritius, learned from exposure experiments that *B.*(*P.*) *forskali* was more attractive to miracidia than other snails common on the island. Repeated attempts resulted in successful transmission of *S. haematobium* on an experimental basis. These findings subsequently aroused concern for *B.*(*P.*) *forskali* in different parts of *Africa*. Buckley's (1946) attempt to infect this snail in Northern Rhodesia were unsuccessful. Others such as Ransford (1948) in Nyasaland and Schwetz (1951b) in Uganda, on the assumption that this snail might be a vector, made searches in the field examining hundreds of specimens for infection. In both cases, the searches were negative.

Attempts were made by the Bilharzia Snail Control Section (Barlow) at Cairo to infect the local *B.*(*P.*) *forskali* with the Egyptian *S. haematobium* but these met with failure. Further attempts along this line were also made in Cairo by the present author (unpublished data). No cercariae but small poorly developed sporocysts were found in six of 700 laboratory-reared *B.*(*P.*) *forskali* when crushed 8–15 days after exposure to miracidia of *S. haematobium*. Continued work on this project at NAMRU-3 has resulted in the production of cercariae by one snail of approximately 1500 exposed to *S. haematobium* (Wells, 1954). Examination of 1466 *B.*(*P.*) *forskali* from the field in areas endemic for *S. haematobium* revealed seven infected snails (El Gindy, 1955). Mice infected with cercariae from these snails produced five male schistosomes identified as *S. haematobium*. LeRoux (1954a) reported successful infection of *B.*(*P.*) *forskali* from Gambia with *S. haematobium* of Egyptian origin.

Elsewhere *B.*(*P.*) *forskali* seems to be more receptive to infection as indicated by de Meillon (correspondence to Gisman, 1954) who obtained three infected

snails from 37 exposed to *S. haematobium*. Cowper (1953b) in studying the epidemiology of schistosomiasis haematobia in Mauritius confirmed the earlier reports of Adams (1934, 1935) but was unable to find naturally infected *B.(P.) forskali* or other snails occurring in endemic areas. LeRoux (1954b) as a result of studies of snails in their habitats on Mauritius did not necessarily accept *B.(P.) forskali* as the vector of *S. haematobium* on this island but felt that another snail may be involved. He stated that he discovered the so-called "*Bulinus forskali*" naturally infected with mammalian schistosome cercariae at three separate localities. It is of additional interest to note that Dr. LeRoux also found mammalian schistosome cercariae in *B.(P.) forskali* at Kisunu, Kenya. Adult female schistosomes obtained from mice infected with cercariae from this snail in the same area possessed eggs which resembled those of *S. bovis*. Also, eggs resembling *S. bovis* were obtained from a goat in the same area. Certainly these situations emphasize the significance that an understanding of parasite-snail relationship bear to studies of the schistosome complex and to the epidemiological aspects of schistosomiasis.

Apparently the schistosomes of birds and lower mammals, some of which are likely to be the causative agents for dermatitis in man, have been given only passing attention. The following list includes the more common schistosomes reported for man and animals in Africa and the Middle East. The identification and taxonomic status of several species is questionable and must await further investigation before an acceptable taxonomic scheme can be proposed; *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858; *S. bovis* (Sonsino, 1876) Blanchard, 1895; *S. intercalatum* Fisher, 1934; *S. matthei* Veglia and LeRoux, 1929; *S. spindalis* Montgomery, 1906; *S. spindalis* var. *africana* Porter, 1926; *S. curassoni* Brumpt, 1931; *S. mansoni* Sambon, 1907; *S. mansoni* var. *rodentorum* Schwetz, 1951; *S. rodhaini* Brumpt, 1931; *S. faradjei* Walkiers, 1928; *S. magrebowiei* LeRoux, 1933; *S. turkestanicum* Skrjabin, 1913; *Bilharziella polonica* (Kowalewski, 1895) Looss, 1899; *Bivitellobilharzia loxodontae* Vogel and Minning, 1940 and *Schistosomatium pathlocopticum* Tanabe, 1923.

Strangely enough in Africa, a continent confronted with a multitude of problems in schistosomiasis, there has been little study given to the subject of dermatitis or to animal schistosomes that cause it. Generalized dermatitis of the abdomen and legs is of frequent occurrence in fishermen and farmers who spend hours each day in the water of irrigation canals in areas where *S. mansoni* and *S. haematobium* are endemic. Natives are aware of water-borne schistosome dermatitis and some even have a general idea of its cause or origin. Some villagers have been sufficiently impressed by the severity of schistosome dermatitis as to take special precaution to avoid parts of canals in which it has been reported as prevalent.

Schistosome dermatitis as a problem of medical interest to the welfare of the people has not been evaluated in Africa. Wright and Roberts (1944) described a case of creeping eruption, presumably caused by invasion by *S. mansoni*, in a boy in Kenya. Dr. Barlow (Barlow and Meleney, 1949) by self infection has demonstrated the obvious dermatitis that follows penetration of skin by the

cercariae of *S. haematobium* in Egypt. Cattle raisers in the Sudan have noted dermatitis in cows as well as in the cattle herders after contact with waters of certain canals. Fairley (1951) stated that dermatitis resulted from human infection by *S. bovis*. Dermatitis was reported by Raper (1951) who experienced an accidental infection by this parasite.

Undoubtedly there are numerous avian and mammalian schistosomes responsible for dermatitis of man in Africa and the Middle East but this problem by comparison is not sufficiently important to demand studies along such lines at this time.

PORTUGAL

Schistosomiasis in Portugal is not as serious as in most other countries where the disease occurs. The first case was reported by Borges (1921) in the Province of Algarve. Infections have been recorded subsequently in other villages but the parasite has not spread as rapidly or as far as might be expected, knowing that the snail vector is a fairly common mollusc in waters where people habitually bathe, launder clothing and collect water for household uses. Thus far the majority of infections have occurred in young girls and women washing clothes in bodies of water fed by warm springs.

Schistosomiasis resulting from infection by *S. haematobium* in Portugal is unique and constitutes an unusual niche in our present day understanding of this disease. This schistosome is morphologically similar to *S. haematobium* which is found throughout the greater part of Africa where *Bulinus* and *Physopsis* serve as transmitters. In Portugal, this parasite is restricted in its distribution and is transmitted by *Planorbis dufourii* (Fraga de Azevedo and Colaço, 1950). Taxonomic status of the snail host is still in dispute. It has been referred to variously as *Planorbis corneus* var. *metidjensis*, *P. metidjensis* var. *dufourii*, *P.* (*Planorbis*) *dufourii* (Baylis, 1931) and more recently by Amberson and Schwarz (1953) as *Planorbarius corneus metidjensis*.

Apparently Bettencourt and Borges (1922) were the first to obtain adult *S. haematobium* of Portuguese origin in experimentally infected mice. Fraga de Azevedo *et al.* (1948) experimentally infected monkeys (*Cercopithecus*) which showed lesions in the liver and intestinal walls but eggs were never found in the urine. Fraga de Azevedo and Colaço (1950) compared *S. haematobium* of Portugal obtained from experimentally infected hamsters and monkeys with *S. haematobium* of Egypt and South Africa. Adult worms from the different areas were similar but there was considerable range in their size, which the authors attributed to the fact that the Portuguese material had been reared in experimental hosts. In this respect it is of interest to note that the schistosomes from hamsters were larger than those found in primates, a feature reported also for experimental infections with *S. haematobium* in Egypt (Kuntz and Malakatis, 1955a). A comparison of egg measurements of *S. haematobium* from different regions revealed that those from *Portugal* were slightly larger than eggs from the same parasite in Egypt and South Africa but the average length of the spine was shorter.

The cercaria of *S. haematobium* in Portugal was described in detail by Bettencourt and da Silva in 1922. Blacklock and Thompson (1924), working with *S. haematobium* in Sierra Leone, found the morphology of the cercaria there quite different from that of Bettencourt and da Silva and they intimated doubts regarding the Portuguese schistosome and the fact that it was reported from a *Planorbis*. The latter authors (1925) reconfirmed their previous observations after a careful study of much additional material. They maintained that the cercaria of *S. haematobium* in Portugal possessed three pairs of flame cells in the body as well as three pairs of penetration glands. These features, if constant, would allow for differentiation of this schistosome from the cercariae of those presently known in Africa since the latter seem to be characterized by five pairs of penetration glands and four pairs of flame cells in the body. However, as already intimated in the discussion on Africa it seems that attempts to distinguish species of schistosomes by their cercariae is somewhat hazardous with our existing knowledge and contradictions of cercarial morphology.

In their early studies Bettencourt *et al.* (1921) successfully infected *P. dufourii* from different localities in Portugal with the miracidia of *S. haematobium*. França (1921), pursuing similar interests, observed that miracidia were definitely attracted to *P. dufourii* and stated that he had seen many miracidia entering the snail. The majority of infected snails died and developing sporocysts were found in the body.

Unfortunately, there has been little investigational inquiry elucidating the possible parasite-snail relationships of the Portuguese *S. haematobium* to molluscs from other geographical areas. Carrosse (1930), using *S. haematobium* eggs from infected Senegalese soldiers, attempted to infect various snails in Morocco. He reported success in infection of *Bulinus*, but not the local *P. dufourii*. Dr. C. H. Barlow (Gisman, 1954) while in Cairo attempted to infect *P. dufourii* sent to him from Portugal with the Egyptian *S. haematobium*. Apparently, there was some attraction for the miracidia, but there was no evidence of snail infection.

Although *P. dufourii*, presumably identical with that of Portugal, occurs in the northwestern part of North Africa, there is no evidence that this snail is naturally infected in regions where *Bulinus* is the known vector of *S. haematobium*. Obviously in this instance as well as in others, there is need for careful experimental work on snail infections following a standardized procedure to indicate relationships of snails and the schistosome parasite of Portugal with their counterparts in Africa.

In the translations available, there was no indication of dermatitis resulting from invasion by *S. haematobium* or by schistosomes of birds or lower mammals in Portugal. There have been numerous reports of dermatitis of schistosome origin in employees of fish hatcheries and others working in fresh waters in France, Germany and Switzerland and a number of dermatitis-producing cercariae have been described from different parts of Europe and from England (Cort, 1950). Salomé (1954) has recently reported schistosome dermatitis in the Netherlands.

INDIA

In its present status, schistosomiasis in India is primarily a disease of concern in domestic livestock and occasionally in lower mammals. India lays claim to more schistosomes than many other countries; Dutt and Srivastava (1952) state that six of 15 known valid species of mammalian schistosomes have been described from their country. Mukerji *et al.* (1946) report that there have been at least five cases of supposed schistosomiasis haematobia reported in individuals who had never been outside India. The possibility of human schistosomiasis being established in India has been a point in discussion for a number of years. India, during the periods of World Wars I and II, and since, has not failed to recognize the potential danger of housing infected troops from Africa within her borders.

There may be justification for more concern about human infection as a result of the discovery of an endemic focus of urinary schistosomiasis by Gadgil and Shah (1952) in the Ratnagiri district of Bombay State. They detected 250 cases of schistosomiasis in a village with a population of 1200. Terminally-spined eggs reported as uniform in all cases and morphologically resembling those of *S. haematobium* were recovered from the urine of persons whose only major symptom was hematuria. Evidence presented indicated an unusual parasite-host compatibility in which there was little discomfort and disability among infected natives. The authors assumed that there was recovery from infection at middle age since they found it difficult to demonstrate eggs in urine samples, hematuria had subsided and elderly persons were free from complaint although they gave histories of having been infected at an earlier age.

Approximately 2.6 per cent of snails, *Paludomus obesa* (Melanidae), taken in the area were infected with cercariae reportedly indistinguishable from those of *S. haematobium*. In recent correspondence Dr. Gadgil (1954) indicated that he had not been able to procure adult parasites from experimentally infected animals.

Previous evidence was of such a nature as to suggest that cases reported as autochthonous for India probably represented rare or accidental infection of man by one of the schistosomes of animals which produce eggs similar to those of *S. haematobium*. The situation as reported by Gadgil and Shah (l.c.) changes the status of schistosomiasis in India and possibly indicates the recognition or even the development of a human-lower-mammal schistosome complex not unlike those arousing current interest in Africa. In accordance with our present knowledge of the snail hosts that usually transmit the mammalian schistosomes, it would indeed be very surprising to learn that a snail of the family Melanidae would serve this function.

Although the schistosomes of livestock in India have not been studied experimentally on such a scale as the schistosomes of Africa and America, there is ample evidence that these species are capable of infecting mammals other than their usual definitive hosts. A few illustrations may be cited. Buckley (1938) recovered adult *S. spindalis* from mice exposed to cercariae responsible for paddy itch in Malaya. *S. indicum*, usually considered as a parasite of horses, buffalo, goats and sheep in India, was reported from dogs and other animals. Khaw (1947)

obtained *S. indicum* from rabbits exposed to cercariae from *Indoplanorbis exustus* and *S. suis*, a parasite of pigs and a cause of canine schistosomiasis, from cercariae which emerged from *Lymnaea*. Srivastava and Dutt (1951) infected guinea pigs but the parasites were not as well developed as those recovered from kid goats and no eggs were found in paired females. *Ornithobilharzia dattai* was described by Dutt and Srivastava (1952) from parasites obtained from guinea pigs and a water buffalo. Fairley (1927) attempted to infect *Macacus sinicus* with *S. spindalis* but failed since the infection progressed to a certain stage then died out. Apparently in India there has been no use of rodents which have served so well as hosts for experimental schistosomiasis in other countries.

There are at least two schools of thought with reference to the taxonomic status of schistosomes in India. Alwar in 1950 stated that only five species (*S. nasalis*, *S. spindalis*, *S. indicum*, *S. suis* and *S. nairi*) have been definitely proven to occur in India. Listed as doubtful were *S. bovis*, *S. haematobium*, *S. japonicum* and *Ornithobilharzia bomfordi*. As in Africa, identification of the schistosomes has been uncertain because of numerous reports based on ill-defined infections in which adult worms were not obtained or the presence of a parasite was judged upon the egg alone. Although there have been several records of *S. bovis* in India, both Rao (1939) and Bhalerao (1947) agreed that it is non-existent. It has been the general consensus of opinion that *S. haematobium* does not occur in that country; yet Moghe (1945), in a survey of livestock, listed both *S. bovis* and *S. haematobium* as parasites of sheep, goats and cattle.

Schistosomes collected from pigs and in an advanced stage of decomposition were identified by Bhalerao (1934) as *S. japonicum*. Later it was agreed however that these schistosomes were the same as those described as *S. suis* (Rao and Ayyer, 1933) (syn *S. incognitum*), possessing eggs identical with those upon which Chandler (1926) had based his description of *S. incognitum*. *S. spindalis* and *S. nasalis* also are very similar species, differentiation being based upon slight differences in body characteristics of adults and cercariae, and principally upon the location of the parasite in the definitive host. *S. nasalis* is commonly found in the nasal passages and is responsible for the "snoring disease" of cattle.

Studies of cercariae, though limited in number, have played a part in an understanding of the biology of the schistosomes in India. The cercarial stage of *S. spindalis*, the first cercaria of an animal schistosome to be recognized, was identified after Liston and Soparkar (1918) obtained adult worms from experimentally infected animals. Morphologically it resembled that of Sewell's (1922) *Cercaria Indicae XXX*. Both cercariae were equipped with the usual five pairs of penetration glands but *S. spindalis* was different in possessing four rather than three pairs of flame cells in the body. Although the value of the number of cells in the excretory system as a criterion for differentiation may be questioned, Rao (1938), after he had demonstrated *S. nasalis* to be the adult stage of *Cercariae Indicae XXX*, used the presence of an additional pair of flame cells as one of several differentiating features to separate it from the closely related *S. spindalis*. In a study of the life cycle of *S. indicum* Srivastava and Dutt (1951) described the cercaria with five penetration glands and four pairs of flame cells

in the body. They also revealed that the cercaria of *Ornithobilharzia dattai* possessed five penetration glands but only three pairs of flame cells in the body. A difference in flame cell count was then suggested as one of several points of differentiation to distinguish this cercaria from certain other species of schistosomes. In India as elsewhere much remains to be learned if a satisfactory scheme for recognition of larval schistosomes is to be proposed.

The return of troops who had contracted schistosomiasis in Egypt during World War I and the presence of infected Africans in India during the past war aroused some apprehension and concern that this disease might be introduced into the country. Steps were taken to determine whether snails in areas occupied by troops previously infected with *S. haematobium* were capable of serving as transmitters. A series of snails including *Lymnaea* and *Indoplanorbis*, now listed as vectors for several of the animal schistosomes, were exposed to miracidia from infected Indians (Kemp and Gravely, 1919). The results were negative. Sewell (1919), describing the cercariae of India, found one, *Cercaria Indicae XXX*, in *Indoplanorbis exustus*, which was of some concern since it was similar to *S. japonicum*. Rao (1938) by experimental infection showed Sewell's cercaria to be *S. nasalis*. In 1946, Mukerji *et al.* repeated the earlier investigations by Kemp and Gravely (l.c.) but exposed local molluscs to miracidia of *S. mansoni* as well as *S. haematobium*. Negative results by Mukerji and by Khaw (1947) engaged in a similar study, and the fact that the latter failed to find appropriate vectors indicated the improbability of human schistosomiasis occurring in India. Brumpt (1936) attempted unsuccessfully to infect *Indoplanorbis exustus* with *S. mansoni* and *S. bovis*. Similar exposures of miracidia of *S. mansoni* and *S. haematobium* to snails in Ceylon, including *I. exustus*, were also negative (Sivalingam, 1949).

Although the taxonomic status is very unstable, the following species of schistosomes, recognized by various investigators, may be listed for India: *Schistosoma indicum* Montgomery, 1906; *S. spindalis* Montgomery, 1906; *S. incognitum* Chandler, 1926; *S. suis* Rao and Ayyer, 1933; *S. nasalis* Rao, 1932; *Ornithobilharzia bomfordi* (Montgomery, 1906) Price, 1929; *O. nairi* (Mudalair and Ramanujachan, 1945) Bhalerao, 1947; *O. dattai* Dutt and Srivastava, 1952; and *Gigantobilharzia egreta* Lal, 1937. All except the last named species, which is found in birds are parasites of mammals.

Neither the medical nor the veterinary professions have made any issue of schistosome dermatitis in India. Certainly such conditions must prevail in a country in which so many of the livestock and snails are infected with different species of schistosomes. Dermatitis undoubtedly occurs but probably has not been distinguished from general types of skin irritation or given cognizance in peoples affected by more serious medical problems. Bhalerao (1948) made rather indefinite statements concerning schistosome dermatitis in men bathing in fresh water tanks in the Mysore State. deSa and Monteiro (1949) did not cite specific cases but commented that animal schistosomes probably were responsible for dermatitis in India. In Malaya, Buckley (1938) attributed swamp or rice paddy itch of man to cercarial penetration by *S. spindalis* of the water buffalo. Derma-

titis of cercarial origin probably will gain recognition in India at a later date after additional studies reveal more of the basic biology of the schistosomes of lower mammals and birds.

AMERICAS

It is generally accepted that *Schistosoma mansoni* was primarily a parasite of man in Africa but that it became established in northern South America and parts of the West Indies as a result of the importation of slaves. Schistosomiasis, though recognized in the American tropics for a number of years, has only recently been considered in greater detail by investigators interested in its biological and epidemiological aspects. In the light of our present knowledge a single species of schistosome, *S. mansoni*, does not necessarily imply a state of simplicity if one is cognizant of the many details upon which the complete story of the biology of the schistosome is based. Numerous researches have demonstrated that schistosome problems in the western hemisphere are not necessarily restricted to tropical regions. Increasing respect and recognition have been given to the animal schistosomes responsible for cercarial dermatitis occurring in widely spread areas, especially in the northern parts of North America.

In the Americas as elsewhere it was generally assumed for some time that man or primates were the only definitive hosts for *S. mansoni*. The non-specificity for definitive hosts of *S. mansoni* of the western hemisphere has been adequately illustrated (Moore *et al.*, 1949; Stirewalt *et al.*, 1951). Mammals subjected to experimental infection have shown varying degrees of compatibility from suitable parasite-host relationships in mice and hamsters to incompatibility in dogs (Stirewalt *et al.*, l.c.). There may be exceptions to the latter since Pinto and de Almeida (1945) reported infection of pups in Brazil. *S. mansoni* is readily adaptable to rodents, especially mice and hamsters, and there is no need for a gradual transition from human to the rodent hosts (Moore and Meleney, 1952a). Successful infection of laboratory mammals was followed by exposure of wild rodents to schistosome cercariae. Moore and Meleney (1952b) infected the rice rat, *Oryzomys palustris*, from Florida with *S. mansoni* and recovered viable eggs from the feces. The authors mentioned the possibility of such hosts serving as reservoirs.

In earlier attempts to infect various animals, Pinto (1944) obtained negative results with a pig but successfully infected a laboratory-raised hairy armadillo (*Euphractus*) with *S. mansoni*. Ruiz listed the crab-eating raccoon (*Procyon*) (1952) as well as the pacca (*Cuniculus*) and the grison (*Grison*) as experimental hosts in Brazil (1953). Travassos (1953) infected an opossum (*Didelphis*). In the same year, de Amorim (1953), in examining wild animals for use in susceptibility work, discovered the rodents *Nectomys*, *Oxymycterus* and *Holochilus* naturally infected and later (1953) added *Zygodontomys* and *Oryzomys*. *Rattus rattus frugivorus* and *Cercomys* were infected in the laboratory. The authors speculated on the role of certain rodents in the close association of people, rats, parasites and planorbid snails. *Rattus r. frugivorus* was found naturally infected by Barbosa *et al.* (1953).

These results serve to indicate how susceptibility studies followed by field work have unveiled rather basic and important factors in the epidemiology of schistosomiasis. It will be of interest to follow the progress of such studies to learn whether additional observations will parallel those in Africa, giving recognition to rodent strains or subspecies of *S. mansoni*.

As in previous discussions, the snail infection studies of American schistosomiasis have added far more to the recognition of the complexity of the schistosome story than did the definitive host studies. This field of inquiry was initiated by the research of Vogel (1942) in which it was shown that the susceptibility of planorbid snail hosts from West Africa was different from that of snail hosts of South America when both were exposed to infection by *S. mansoni* of West African origin.

Subsequent researches involved different types of snail infection experiments in which it was demonstrated that *Tropicorbis* of Louisiana possessed a low grade and variable susceptibility for *S. mansoni* under conditions conducive to infection (Cram, *et al.*, 1945, 1946, 1947). *Tropicorbis havanensis* from Baton Rouge were susceptible to Puerto Rican *S. mansoni* whereas *Tropicorbis* collected from New Orleans were refractory. McQuay (1952) confirmed the observations of Cram *et al.* (*l.c.*) and further called attention to the suggestion of wide differences in the susceptibility of *T. havanensis* tested from different parts of Louisiana. Brooks (1953) in the same laboratory carried the problem a step further and discovered that miracidia of the Puerto Rican *S. mansoni* penetrated *T. havanensis* from Baton Rouge and *Australorbis glabratus*, the natural host in Puerto Rico, equally well. Miracidia having gained entry into the snail host evoked a definite tissue response in *T. havanensis* but not in *A. glabratus*. Brooks concluded that successful development of the schistosome in its snail host may be due more to intrinsic variation in the miracidia than to their chance location in exceptionally susceptible individual snails. A similar relationship seems to exist for the schistosome miracidia which penetrate susceptible and nonsusceptible strains of *A. glabratus*. Newton (1952) observed that the miracidia of *S. mansoni* apparently develop without host interference in the susceptible *A. glabratus* from Puerto Rico whereas in the nonsusceptible strain from Brazil the parasite, a day or two after penetration, succumbs as a result of a definite tissue response and a fibrotic type of walling-off reaction. Certainly these disclosures support the contention of this and other investigators that the mere penetration of a snail by miracidia cannot be used as a criterion of susceptibility.

One of the most revealing studies was that of Files (1951) which dealt with the susceptibilities of snail hosts to five strains of *S. mansoni* originating from Puerto Rico, Venezuela, Surinam, Brazil and Egypt. Seven additional cross strains of the parasite were established by crossing strains from the above listed countries. Miracidia from the different strains of *S. mansoni* were used for infection of *Australorbis glabratus* from different parts of northern South America and the Caribbean area, *Biomphalaria pfeifferi* from Liberia and *B. boissyi* from Egypt. As a result of various combinations, Files demonstrated that snail vectors as well as the schistosome parasites showed definite physiologic differences between

continents or even between areas of one country. In the words of the author, instances of incompatibility between vectors and parasites of different endemic areas may be explained by "(1) interspecific and intraspecific differences between vectors in respect to the physiological factors that are responsible for development of the parasite in the snail, and (2) physiological differences between the strains of the parasite".

Recognition of physiologic differences indicating the existence of strains of parasite and snail host suggested the possibility that genetic factors might be involved (Newton, 1953). As a result of a carefully planned study, Newton successfully demonstrated that the susceptibility of *A. glabratus* to infection by *S. mansoni* was a heritable character. Newton gave due consideration to intrinsic and extrinsic factors of "nongenetic" nature which could directly influence the outcome of a study involving genetics. On the other hand McQuay's (1953) investigations indicated that the low grade susceptibility of *Tropicorbis havanensis* to infection with *S. mansoni* of Puerto Rican origin was not notably increased by inbreeding of selected susceptible stocks of snails.

Certainly these searches into the smaller facets of the schistosome's make-up introduce new fields of thought with regard to the parasite-snail and the parasite-definitive-host aspects of schistosomiasis.

Although only one schistosome of man is known from the Americas, there is disagreement on the morphological features of its cercarial stage. According to Faust and Hoffman (1934), Iturbe and Gonzalez showed only three pairs of penetration glands for the cercaria of *S. mansoni* in Venezuela. Faust and Hoffman, and later Maldonado and Matienzo (1947), described six pairs of penetration glands and four pairs of flame cells in the cercaria from Puerto Rico. Kuntz (1950), however, observed only five pairs of penetration glands in mature cercariae from Puerto Rico but found five pairs of flame cells. As suggested by Faust and Hoffman (l.c.) it seems that there may be two factors which account for such discrepancies, "either the cercaria of *S. mansoni* is extremely variable in its internal anatomy or human observations are equally variable." Another factor accounting for discrepancies is the failure of authors to stage the conditions under which cercariae were studied, i.e., whether they were removed from crushed snails or were mature and obtained after natural emergence from the snail host.

The majority of schistosomes described from the Americas are parasites of birds. *Schistosomatium douthitti* (Cort, 1914. Syn. *S. pathlocopticum* Tanabe, 1923) is a parasite of water-frequenting and lowland mammals and *Heterobilharzia americana* Price 1929 has been reported from a raccoon and a *Lynx*. The list of schistosomes from birds includes: *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953; *Bilharziella polonica* (Kowalewski, 1895) Looss, 1899; *Dendritobilharzia anatinarum* Cheatum, 1941; *Gigantobilharzia gyrauli* (Brackett, 1940) Brackett, 1942; *G. lawayi* Brackett, 1942; *G. huronensis Najim*, 1950; *Macrobilharzia macrobilharzia* (Travassos, 1923) Price, 1929; *M. anhingae* (Price, 1929) Price, 1931; *M. chapini* Price, 1929, (syn. *A. variglandis* Miller and Northup, 1926); *M. manitobensis* McLeod, 1936; *M. canadensis* McLeod,

1936; *M. lari* (syn. *Ornithobilharzia lari*) McLeod, 1937; *Ornithobilharzia pricei* Wetzel, 1930; *O. filamenta* McLeod, 1940; *O. aviani* McLeod, 1940; *Trichobilharzia cameroni* Wu, 1953; *T. physellae* (Talbot, 1936) McMullen and Beaver, 1945 (syn. *Pseudobilharzia querquedulae* McLeod, 1937); *T. ocellata* (LaValette) McMullen and Beaver, 1945 (syn. *T. kossarewi*, Skrjabin and Zakharow); *T. stagnicolae* (Talbot, 1936) McMullen and Beaver, 1945; *T. burnetti* (Brackett, 1942) McMullen and Beaver, 1945; *T. horiconensis* (Brackett, 1942) McMullen and Brackett, 1945; and *T. waubesensis* (Brackett, 1942) McMullen and Beaver, 1945. According to Penner (1953) *Microbilharzia* Price, 1929 should be considered as a synonym of *Austrobilharzia* Johnston, 1917.

Clinical and biological recognition of schistosome dermatitis has resulted in reports of its occurrence in all parts of the United States. Although schistosome dermatitis has been described in the Caribbean and South America, the majority of cases have been inside the United States and Canada. Cort gave a comprehensive review of dermatitis up to 1950. The subject has assumed greater importance with recent reports of dermatitis-producing cercariae from marine molluscs and with the discovery of schistosome dermatitis in Hawaii and as far north as Alaska.

Since Cort's (1950) report, Penner (1950) described *Cercaria littorinalinae* (adult, *Austrobilharzia*, Penner, 1953) from the marine snail *Littorina* on the coast of Southern California and Stunhard and Hinchliffe (1952) described the life history of *Microbilharzia variglandis* from the mud snail *Nassa* on the coast of Rhode Island. Both produce dermatitis and the latter is responsible for a serious "clam digger's dermatitis" upon invasion of the skin of previously sensitized persons. Chu reported dermatitis-producing marine cercariae from *Littorina* for the first time in Hawaii and recently (Chu and Cutress, 1954) identified it as *A. variglandis* (Miller and Northup, 1926) Penner, 1953 and called attention to suppression of the name *Microbilharzia* by Penner (1953). Hutton (1952) announced the discovery of a schistosome cercaria (*Cercaria huttoni* Leigh 1953) from the marine snail *Haminoea* which was suspected as the cause of seabather's eruption in Florida.

Simonds *et al.* (1951) produced dermatitis experimentally with unidentified cercariae obtained from *Physa* in California. It is now known that there are at least three distinct species of *Trichobilharzia* in the Pacific Northwest (Macy and Moore, 1953) including *T. oregonensis* (*Cercaria oregonensis*) described earlier as a cause of dermatitis. A new schistosome of birds, *T. cameroni*, whose cercarial stage is the causative agent for dermatitis in eastern Canada was described by Wu (1953). Examining the source of dermatitis of children in Florida, Leigh (1954) found cercariae similar to *Trichobilharzia physellae* emerging from *Physa*.

Experimentally, Olivier (1953) demonstrated that the dermatitis-producing cercariae of *Trichobilharzia stagnicolae*, *T. physallae* and *T. ocellata* were capable of penetrating the skin of mice and that the young schistosomes could cause pulmonary lesions after migration to the lungs. Olivier and Weinstein (1953) further demonstrated that rabbits sensitized by repeated exposure to cercariae of *T. stagnicolae* and *T. ocellata* developed dermatitis similar to that in man.

Although Canada with its many lakes and breeding grounds for migrating birds is outstanding for schistosome dermatitis, it is of interest to state that the range of dermatitis has been extended to the interior of Alaska. Harkema (1953) found a dermatitis-producing *Cercaria* sp. in two species of *Lymnaea*. Jarcho and van Burkalow (1952) made an interesting comparison of the distribution of schistosome dermatitis and various contributing factors such as distribution of snail carriers in the glaciated lake region of North America and the flyway of the blue-winged teal, one of the definitive hosts for the dermatitis-producing schistosome cercariae. They have indicated that man is added as a part to the parasite-snail-bird association necessary for the completion of a pathogenic complex resulting in dermatitis.

ORIENT

Unusual biological characteristics such as the almost unlimited ability of *Schistosoma japonicum* to parasitize effectively a wide range of mammals and the fact that it is transmitted by amphibious snails separate this schistosome from all others found in man and domestic animals. Oriental schistosomiasis in man and lower mammals occurs in widely separated areas of a large geographical region yet only one species of *Schistosoma* has been incriminated as the causative agent and so far as known all vectors are species of a single genus of snails (*Oncomelania*).

In spite of the fact that biological studies of *S. japonicum* have been concerned with the occurrence of this parasite in naturally infected animals, it seems that there has been no compelling urge on the part of investigators to describe new species or to designate strains. Meleney and Faust (1923) in their search for snail hosts of *S. japonicum* in China, however, took advantage of an opportunity to make cross infection experiments to determine whether the *Schistosoma* of China was identical with that of Japan. The Japanese intermediate host was exposed to miracidia from a Chinese patient and the Chinese snail to the miracidia from a dog with *S. japonicum* of Japanese origin. As a result, the biological similarity of the two representatives of *S. japonicum* was confirmed. Bonne *et al.* (1942) obtained *S. japonicum* from dogs, deer and man in the Celebes but found no differences in the adult parasites. After a demonstration of the significance of cattle as natural reservoirs for schistosomiasis in China Wu (1938) made the assumption that the disease in cattle and man was essentially the same and he stated that the existence of "physiological strains" of *S. japonicum* in cattle and man was unlikely.

The latest information provided by the parasite-snail infection researches of DeWitt (1954) and the parasite-definitive-host studies by Hsü and colleagues (1955a; 1955b; 1955c) obviously alter the trend of thought with regard to the probable occurrence of geographic and/or physiologic strains of *S. japonicum*. Furthermore, the work of Hsü *et al.* which has emphasized the enzootic nature of schistosomiasis in Formosa suggests that the physiologic strains may be subdivided into lower mammalian and human categories.

Recognition of the general susceptibility of mammals to infection by

S. japonicum and the use of these animals in early studies of the biology have made later systematic evaluation of small mammals as hosts for experimental purposes unnecessary. However, the ability of *S. japonicum* to establish itself in a number of hosts was indicated in the papers of Magath and Mathieson (1945, 1946) describing infection of pigs, dogs and wild rats in the Philippine Islands, by the list of Wright (1950) based upon the examination of mammals in Japan and more recently by domestic animals and many rodents found infected by Hsü *et al.* (l.c.) in Formosa. It is still debatable in the minds of some that the infection as seen in these various hosts represents a truly compatible parasite-host relationship which materially aids in propagation of schistosomiasis. Magath and Mathieson (l.c.) found eggs in the feces of only a few of the rsat trapped in the Philippines, so concluded that they probably contribute little or nothing to the life history of the parasite. Wright (l.c.) on the other hand, indicated that reservoir hosts among lower mammals contribute to the spread of schistosome infection in Japan, and Wu (1938) came to a similar conclusion in China.

Extensive studies by Hsü and colleagues (1955a; 1955b) in Formosa favor the contention that the *S. japonicum* infection of mammals is of such a nature that it can and does play an important role in the propagation of schistosomiasis. The survey of Hsü *et al.* (1955a) showed that five of nine species of small mammals (*Rattus rattus*, *R. norvegicus*, *R. losea*, *Mus formosana* and *Crocidura murina*) examined were naturally infected, the incidence ranging from 21.5 per cent for *R. norvegicus* to 8.7 per cent for *R. rattus*. Fecal examination revealed the presence of viable eggs in a large proportion of hosts trapped. Additional concern was expressed for the insectivore, *Crocidura*, since it was the most abundant and the incidence of infection indicated that it probably played an important role. It is of particular significance to note that infected snails were obtained from waters more or less inaccessible to man or domestic animals, thus implying that the lower mammals are responsible for maintenance of schistosome infection in certain localities.

Schistosomiasis japonica in Formosa, presumably, is a disease of livestock and lower mammals and one in which man plays only a questionable role. These and other findings by Hsü and his colleagues have introduced the suggestion of a "non-human" strain of *S. japonicum* or even a "canine strain" which may account for the high percentages of infection reported for dogs. A survey (Hsü *et al.*, 1955c?) in which 4,197 people were examined from seven villages revealed no positive cases of *S. japonicum* infection.

Field studies in Formosa were augmented with attempts to infect both human volunteers and primates with *S. japonicum*. Five volunteers previously exposed to 500 to 800 cercariae each passed no eggs and showed no evidence of schistosome infection in liver biopsies at the end of the incubation period. There were no definite diagnostic symptoms of infection, although eosinophilia and positive intradermal reactions were recognized. It is much too early to form definite conclusions with respect to the different geographic relationships but Dr. Hsü's observations are of utmost interest. The Formosan monkey, *Macacus cyclopis*, was difficult to infect with the non-human strain of *S. japonicum*, although it

could be easily infected with cercariae from *O. nosophora* from Japan and *O. quadrasi* from the Philippines. The Japanese monkey *M. fuscatus* and the Philippines monkey, *M. philippinensis* could easily be infected with the Formosan *S. japonicum*.

As indicated by Faust and Meleney (1924), the early studies on the morphology of the cercaria of the blood fluke were worked out by the Japanese but the authors agreed with the thoroughly accurate accounts of Cort (1919) describing the cercaria and stated that it corresponded in all details with their observations on the cercariae of Japanese as well as Chinese origin. Apparently no one has disputed Cort's original description.

Until the past few years there has been little incentive for investigations attempting to determine the susceptibility of various snails to infection by *S. japonicum*. The first of these efforts were by Faust and Meleney (1924) who unsuccessfully attempted to infect *Vivipara* and *Lymnaea*. Hunter *et al.* (1949) exposed several genera of snails (*Blandfordia*, *Tricula* and *Fukuia*) from the Fukui and Ishikawa Prefectures of Japan to the miracidia of *S. japonicum* but obtained no infections.

More recent studies, though limited in number, have been very fruitful. The fact that the American snail *Pomatiopsis* and the Oriental *Oncomelania* are closely related led to attempts to infect the former experimentally by *S. japonicm* maintained in laboratory animals in the United States. Stunkard (1946), after exposure of *Pomatiopsis* to miracidia from a dog infected with Philippine Islands *S. japonicum*, found only sporocysts. Likewise Ward *et al.* (1947) found only sporocysts in wild *Pomatiopsis* exposed to miracidia of Chinese origin. Berry and Rue (1948) were more successful in their experimental infections and found mature cercariae in *Pomatiopsis* exposed to *S. japonicum* miracidia of Japanese origin. These findings indicating possible strain differences (Cram, 1953) were further substantiated by the snail infection experiments of Hunter *et al.* (1952), in which it was clearly demonstrated that the *Oncomelania formosana*, vector of *S. japonicum* in Formosa, was not a suitable host for the same parasite of Japanese origin. On the contrary, miracidia of the latter easily infected *O. nosophora* from Japan. Researches along much the same line in the United States by DeWitt (1954) disclosed even more detailed inter-relationships strongly suggesting the probability of geographic strains or basic physiologic differences between *S. japonicum* of different endemic areas. Laboratory-reared *Oncomelania* from different countries were exposed to miracidia of *S. japonicum* from three distinct areas. Different combinations in infection crosses indicated: (1) that *O. hupensis* from Shanghai was readily infected by Chinese and Japanese strains of *S. japonicum* but not with the Formosan strain; (2) *O. nosophora* from Kufu, Japan was susceptible to both Japanese and Formosan strains but was refractory to the Chinese strain of *S. japonicum*; (3) *O. formosana* was infected with the Formosan *S. japonicum* but was not infected with the Chinese and Japanese strains; and (4) *O. quadrasi* from the Philippines was resistant to development of the Chinese and Japanese strain, but a few snails became infected after exposure to the Formosan strain of *S. japonicum*.

An accumulation of data based upon the susceptibility studies of both the

definitive and intermediate hosts to infection by *S. japonicum* has revealed convincing evidence of the existence of Oriental schistosome complexes. At this point, at least with reference to the situation in Formosa, one is faced with a problem similar to that of concern to the investigators in Africa. Is the *S. japonicum* of Formosa one from man that has adapted itself to mammals or is the parasite still in its primitive state, a parasite of lower mammals that has not been introduced into man? Whatever the case may be, it seems likely that the situation in Formosa is not an isolated one and that more detailed regional studies may disclose additional "interspecific" strains or associations within the range of the schistosome now designated only as *S. japonicum*.

There are few reports indicating the presence of other schistosomes in the Oriental fauna. Possibly the failure of recognition of different schistosomes occurring in animals, especially mammals, may be attributed to the assumption by the casual examiner of hosts that all schistosomes encountered are *S. japonicum*. Kuo (1946) searching for bovine schistosomes in China found that *Ornithobilharzia turkestanicum* as well as *S. japonicum* were common in Szechwan. Other schistosomes reported from the Orient include: *Trichobilharzia yokogawai* (Oiso, 1927) McMullen and Beaver, 1945; *T. corvi* (Yamaguti, 1942) McMullen and Beaver, 1945; *Ornithobilharzia odhneri* Faust, 1924; *O. hoepplii* Tang, 1951; and *Gigantobilharzia sturniae* Tanabe, 1948.

Although it seems likely that much of the dermatitis reported as "kabure" in Japan results from cercarial invasion by *S. japonicum*, the actual part played by the human parasite in Oriental dermatitis is a point of dispute. Hunter *et al.* (1952) have shown that the dermatitis known as "koganbyo" (lakeside disease) is caused by the cercariae of *Gigantobilharzia sturniae* in the Shimane Prefecture of Japan. Dermal invasion by these cercariae may be very serious and previously sensitized persons may be incapacitated for several weeks. This schistosome dermatitis, presumably the first reported for Japan, is known to occur in a number of provinces. The adult parasite is a bird schistosome found in starlings, sparrows and wagtails.

SUMMARY

Each geographical region is characterized by schistosomes which infect man or lower animals, or both. In Africa and the Middle East, there are two categories of schistosomes, i.e., those of the *S. haematobium*-type producing terminally-spined eggs and those of the *S. mansoni*-type producing laterally-spined eggs. The parasite-host relationships of the schistosomes is not clearly understood at the present time. Representatives of the *haematobium*-complex, i.e., *S. bovis*, *S. intercalatum*, *S. matthei* and probably others occurring in livestock, are of greater concern in human schistosomiasis. Recent discoveries of schistosomes of the *mansoni*-complex, i.e. *S. rodhaini* and *S. mansoni* var *rodentorum* in rodents and other animals indicate unusual parasite-host relationships with the possibility of concern in the epidemiology of schistosome infections.

Schistosomiasis haematobia in Portugal is unique since the causative agent, *S. haematobium*, is morphologically similar to its counterpart in Africa but is restricted in its distribution and is transmitted by *Planorbis dufourii*.

Although a few autochthonous cases of schistosome infection in man have been reported, schistosomiasis in India at present is primarily a disease of livestock. The status of the schistosome problem in India may change with the recent discovery of an endemic focus of urinary schistosomiasis in the Bombay State.

In the Orient, *S. japonicum* is transmitted by several species of *Oncomelania* and infects man and a wide range of mammalian hosts over a large geographical area. Recent studies indicate the existence of geographical and physiologic strains of the parasite and its intermediate host. On Formosa, contrary to the situation in other areas of the Orient, *S. japonicum* is a parasite of lower mammals, man playing only a questionable role in its propagation. The Formosan schistosome has been designated as a "non-human" strain.

Geographical and physiologic strains of *S. mansoni* and its intermediate hosts in the Americas have been adequately demonstrated. Discovery of wild rodents infected with *S. mansoni* has altered the parasite-host relationships and epidemiological aspects of schistosomiasis in South America.

Schistosome dermatitis has not been differentiated from other types of dermal irritation in countries where the population is concerned with other more serious parasitic infections. In the Americas, however, schistosome dermatitis has received considerable recognition since it is widespread and occurs as a result of dermal invasion of man by animal schistosomes whose cercariae are present in fresh as well as marine waters.

REFERENCES

Anonymous, 1953. Expert committee on bilharziasis: First report, *World Health Organ. Tech. Rpt. Ser.* No. 65: 1–45.

Anonymous, 1954. Bilharzia snail vector identification and classification (Equatorial and South Africa): report of a study-group, *World Health Organ. Tech. Rpt. Ser.* No. 90: 1–22.

Abdel Azim, M., and Cowper, S. G., 1950. On the maintenance of strains of *Schistosoma mansoni*, *S. haematobium* and *S. matthei* in the laboratory in Egypt, with special reference to the use of gerbils, *Brit. J. Exper. Path.* **31**: 577–589.

Adams, A. R. D., 1934. Studies on bilharzia in Mauritius. I. The experimental infection of *Bulinus* (*Pyrogophysa*) *forskali* with *Schistosoma haematobium*, *Ann. Trop. Med. and Parasitol.* **28**: 195–204.

——, 1935. Studies on bilharzia in Mauritius. II. The recovery of adult *Schistosoma haematobium* after development in *Bulinus* (*Pyrgophysa*) *forskali*, *Ann. Trop. Med. and Parasitol.* **29**: 255–260.

Alves, W., 1948. Laboratory meetings, Royal Society of Tropical Medicine and Hygiene. Observations on *S. matthei* and *S. haematobium*. Adults and eggs from experimental animals and man, *Tr. Roy. Soc. Trop. Med. and Hyg.* **41**: 430–431.

——, 1949. The eggs of *Schistosoma bovis*, *S. matthei* and *S. haematobium*, *J. Helminth.* **23**: 127–134.

Alwar, V. S., 1950. A note on schistosomes in India with a review of the literature, *Madras Vet. College Ann.* **6**: 30–35.

Amberson, J. M., and Schwarz, E., 1953. On African schistosomiasis, *Tr. Roy. Soc. Trop. Med. and Hyg.* **47**: 451–502.

Barbosa, F. S., Dobbin, J. E., and Coelho, M. V., 1953. Infestação natural de *Rattus rattus frugivorus* por *Schistosoma mansoni* em Pernambuco, *Publicações Avul. Inst. Aggeu Magalhães* **2**: 43–46.

Barlow, C. H., and Meleney, H. E., 1949. A voluntary infection with *Schistosoma haematobium*, *Am. J. Trop. Med.* **29**: 79–87.

BAYLIS, H. A., 1931. The names of some molluscan hosts of the schistosomes parasitic in man, *Ann. Trop. Med. and Parasitol.* **25**: 369–372.

BECKER, J. G., 1916. A further note on bilharziosis in the Transvaal, *Med. J. South Africa* **12**: 42.

BERRY, E. G., AND RUE, R. E., 1948. *Pomatiopsis lapidaria* (Say), an intermediate host for *Schistosoma japonicum*, *J. Parasitol.* **34** (Suppl.) 15.

BETTENCOURT, A., BORGES, I., AND DE SEABRA, Z., 1921. L'hôte intermédiaire du *Schistosomum haematobium* au Portugal, *Compt. Rend. Soc. Biol.* **85**: 1169–1170.

——, AND BORGES, I., 1922. Le *Planorbis metidjensis*, hôte intermediaire du *Schistosoma haematobium* au Portugal. Confirmation expérimentale, *Compt. Rend. Soc. Biol.* **87**: 1039–1040.

——, ET PEREIRA DA SILVA, E., 1922. Le système excréteur de la cercaire du *Schistosomum haematobium*, *Compt. Rend. Soc. Biol.* **86**: 1050–1052.

——, 1925. The cercariae of *Schistosoma haematobium*, with references to an article of Blacklock and Thompson on human schistosomiasis in Sierra Leone, *Arq. Inst. Bacteriol. Camara Pestana* **6**: 1–21.

BHALERAO, G. D., 1934. On the occurrence of *Schistosoma japonicum* Katsurada in India, *Indian J. Vet. Sc. and Animal Husb.* **4**: 148–151.

——, 1947. Presidential address: Applied helminthology, its past and future in India, *Indian Sc. Congr., Delhi* **34**: 1–20.

——, 1948. Blood-fluke problem in India (review by N. N. de), *Current Sc., Bangalore* **17**: 36–37.

BLACKIE, W. K., 1932. A helminthological survey of Southern Rhodesia, *Mem. Ser. London School Hyg. and Trop. Med.* **5**: 1–91.

BLACKLOCK, D. B., AND THOMPSON, M. G., 1924. Human schistosomiasis due to *S. haematobium* in Sierra Leone, *Ann. Trop. Med. and Parasitol.* **18**: 211–234.

BONNE, C., BORSTLAP, A. J. P., LIE KIAN JOE, MOLENKAMP, W. J. J., AND NANNING, W., 1942. Voortgezet bilharzia onderzoek in Celebes, *Geneesk. Tijdschr. Nederl.-Indië* **82**: 21–36.

BORGES, I., 1921. Un cas autochtone de bilharziose en Portugal, *Bull. Soc. Portugaise Sc. Nat.* **9**: 19–20.

BROOKS, C. P., 1953. A comparative study of *Schistosoma mansoni* in *Tropicorbis havanensis* and *Australorbis glabratus*, *J. Parasitol.* **39**: 159–165.

BRUMPT, E., 1928. L'homme est-il le seul semeur de germes dans le cas de la bilharziose vésicale? *Bull. Acad. Méd., Paris* **100**: 813–818.

——, 1931. Description de deux bilharzies de mammifères africains, *Schistosoma curassoni* sp. inquir. et *Schistosoma rodhaini* n. sp., *Ann. Parasitol.* **9**: 325–328.

——, 1936. *Schistosoma bovis* et *Schistosoma mansoni* ne sont par transmis par *Planorbis* (*Indoplanorbis*) *exustus*. Observations biologiques concernant ce planorbe (autofécondation, érosion de la coquille, élevage, etc.), *Ann. Parasitol.* **14**: 464–471.

—— AND CHEVALLIER, P., 1931. La rate et les splénohépatites des bilharzioses expérimentales, *Ann. Parasitol.* **9**: 15–67.

BUCKLEY, J. C. C., 1938. On a dermatitis in Malaya caused by the cercariae of *Schistosoma spindale* Montgomery, 1906, *J. Helminth.* **16**: 117–120.

——, 1946. A helminthological survey in Northern Rhodesia, *J. Helminth.* **21**: 111–174.

BUTTNER, A., 1953. Sur l'aptitude de certains rongeurs sauvages a devenir réservoirs de virus de la bilharziose intestinale humaine (Note préliminaire), *Bull. Soc. Path. Exot.* **46**: 696–700.

CARROSSE, J., 1930. Enquête sur la bilharziose vésicale (*Schistosoma haematobium*) dans le Sud marocain et la région de Marrakech, *Arch. Inst. Pasteur Algérie* **8**: 90–108.

CAWSTON, F. G., 1921 a. Three schistosomes in Natal which possibly attack man, *J. Trop. Med. and Hyg.* **24**: 242–244.

——, 1921 b. Some South African cercariae, *South African J. Nat. Hist.* **3**: 199–204.

——, 1925. *Schistosomum ? spindalis* Montgomery in Zululand school boy, *J. Trop. Med. and Hyg.* **28**: 406.

CHANDLER, A. C., 1926. A new schistosome infection of man, with notes on other human fluke infections in India, *Indian J. Med. Research* **14**: 179–183.

CHU, G. W. T. C., AND CUTRESS, C. E., 1954. *Austrobilharzia variglandis* (Miller and Northup, 1926), Penner 1953 (Trematoda: Schistosomatidae) in Hawaii with notes on its biology, *J. Parasitol.* **40**: 515–524.

CORT, W. W., 1919. The cercaria of the Japanese blood fluke, *Schistosoma japonicum* Katsurada, *Univ. Calif. Publications Zool.* **18**: 485–507.

——, 1950. Studies on schistosome dermatitis. XI. Status of knowledge after more than twenty years, *Am. J. Hyg.* **52**: 251–307.

COWPER, S. G., 1953 a. Schistosomiasis in Mauritius, *Tr. Roy. Soc. Trop. Med. and Hyg.* **47**: 564–579.

——, 1953 b. The role of the freshwater mollusc, *Bulinus* (*Pyrgophysa*) *forskali* Erhenberg, in the transmission of *Schistosoma haematobium* (Bilharzia disease) in Mauritius, *Proc. Roy. Soc. Arts and Sc., Mauritius* **1**: 259–267.

CRAM, E. B., 1953. Evidence concerning geographic strains of human schistosomes and their molluscan hosts, *Thapar Commemorat. Vol.* 51–62.

——, AND FILES, V. S., 1946. Laboratory studies on the snail host of *Schistosoma mansoni*, *Am. J. Trop. Med.* **26**: 715–720.

——, FILES, V. S., AND JONES, M. F., 1947. Studies on schistosomiasis. Experimental molluscan infection with *Schistosoma mansoni* and *Schistosoma haematobium*, *Natl. Inst. Health Bull.* No. 189, 81–94.

——, JONES, M. F., AND WRIGHT, W. H., 1945. A potential intermediate host of *Schistosoma mansoni*, *Science* **101**: 302–303.

DE AMORIM, J. P., 1953. Infestação experimental e natural de murideos pelo *Schistosoma mansoni*. Nota previa, *Rev. Brasil. Malariol.* **5**: 219–222.

——, DA ROSA, D., AND DE LUCENA, D. T., 1953. Ratos silvestres reservatorios do *Schistosoma mansoni* no nordeste do Brasil, *XI Congr. Brasil. Hig., Curitiba*, (mimeograph) 1–18.

DE MEILLON, B., STOFFBERG, N., AND LURIE, H. L., 1953. Urinary bilharziasis on the Witwatersrand, *South African Med. J.* **27**: 257–258.

DE SA, A. E., AND MONTEIRO, L., 1949. Urinary schistosomiasis in India with a report of one case, *Indian J. Med. Sc.* **3**: 376–381.

DERAMEE, O., THIENPONT, D., FAIN, A., AND JADIN, J., 1953. Sur un foyer de bilharziose canine à *Schistosoma rodhaini* Brumpt au Ruanda-Urundi, *Ann. Soc. Belge Méd. Trop.* **33**: 207–209.

DEWITT, W. B., 1954. Susceptibility of snail vectors to geographic strains of *Schistosoma japonicum*, *J. Parasitol.* **40**: 453–456.

DOWDESWELL, R. M., 1938. Schistosomiasis in the Kavirondo District of Kenya Colony, *Tr. Roy. Soc. Trop. Med. and Hyg.* **31**: 673–688.

DUTT, S. C. AND SRIVASTAVA, H. D., 1952. On the morphology and life history of a new mammalian blood-fluke, *Ornithobilharzia dattai*, n. sp., (Preliminary report), *Parasitology* **42**: 144–150.

EL GINDY, M., 1955. The life cycle of a schistosome liberated from the snail *Pyrgophysa forskali* (Ehrenberg), *J. Egypt. Med. Ass.* (in press).

FAIN, A., 1952. Description de la cercaire de *Schistosoma intercalatum* Fischer 1934 et d'une nouvelle xiphidiocercaire du groupe Ornatae (sous-groupe Prima) *Ann. Soc. Belge Méd. Trop.* **32**: 433–443.

——, THIENPONT, D., HERIN, V., AND DERAMEE, O., 1953. Observations sur *Schistosoma rodhaini* Brumpt au Ruanda-Urundi et description de la cercaire de ce schistosome, *Ann. Soc. Belge Méd. Trop.* **33**: 423–443.

FAIRLEY, N. H., 1927. The early spontaneous cure of bilharziosis (*S. spindalis*) in monkeys (*Macacus sinicus*) and its bearing on species immunity, *Indian J. Med. Research* **14**: 685–700.

——, 1951. Schistosomiasis and some of its problems, *Tr. Roy. Soc. Trop. Med. and Hyg.* **45**: 279–303.

FAUST, E. C. AND MELENEY, H. E., 1924. Studies on schistosomiasis japonica, *Am. J. Hyg. Monogr. Ser.* No. 3: 1–337.

——, AND HOFFMAN, W. A., 1934. Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies 1. The extra-mammalian phases of the life cycle, *Puerto Rico J. Pub. Health and Trop. Med.* 10: 3–49.

FILES, V. S., 1951. A study of the vector-parasite relationships in *Schistosoma mansoni*, *Parasitology* 41: 264–269.

FISHER, A. C., 1934. A study of the schistosomiasis of the Stanleyville district of the Belgian Congo, *Tr. Roy. Soc. Trop. Med. and Hyg.* 28: 277–306.

FRAGA DE AZEVEDO, J., DA SILVA, J. B., DE MATOS COITO, A., COELHO, M. F., AND COLAÇO, A., 1948. O foco português de schistosomíase, *An. Inst. Med. Trop., Lisbon* 5: 175—222.

——, AND COLAÇO, A. F. T., 1950. Sobre a morfologia do *Schistosoma haematobium* de Portugal, *An. Inst. Med. Trop., Lisbon* 7: 7–17.

FRANÇA, C., 1921. A preliminary note on bilharziosis indigenous in Portugal, *Tr. Roy. Soc. Trop. Med. and Hyg.* 15: 180–181.

GADGIL, R. K., AND SHAH, S. N., 1952. Human schistosomiasis in India. Discovery of an edemic focus in the Bombay State, *Indian J. Med. Sc.* 6: 760–763.

——, 1954. Correspondence.

GILLET, J., 1949. Contribution à l'étude de la bilharziose urinaire au Congo Belge, *Ann. Soc. Belge Méd. Trop.* 29: 457–481.

GISMAN, A., 1954. Correspondence.

GORDON, R. M., DAVEY, T. H., AND PEASTON, H., 1934. The transmission of human bilharziasis in Sierra Leone, with an account of the life-cycle of the schistosomes concerned, *S. mansoni* and *S. haematobium*, *Ann. Trop. Med. and Parasitol.* 28: 323–419.

HARKEMA, R., 1953. Study of Alaskan schistosomes, Proj. No. 22-1401-0005, Arctic Aero Med. Lab., Ladd AFB, Alaska 1–26.

HSÜ, H. F., AND LI, S. Y., 1955 a. Studies on schistosomiasis japonica in Formosa. IV. Wild rats and other small mammals as reservoir hosts of *Schistosoma japonicum* in Formosa, (manuscript).

——, LI, S. Y., AND CHU, K. Y., 1955 b. Schistosomiasis japonica among domestic animals in Formosa, G. B. Grassi Commemorat. Vol. *Riv. Parasitol.* (in press).

——, AND RITCHIE, L. S., 1955 c. Epidemiological study on schistosomiasis in Formosa, (manuscript).

HUNTER, G. W., III, AND ABBOTT, R. T., 1949. Studies on potential snails hosts of *Schistosoma japonicum*. II. Infection experiments on amnicolid snails of the genera *Blandfordia Tricula* and *Fukuia*, *Proc. Helm. Soc. Wash.* 16: 86–89.

——, RITCHIE, L. S., AND TANABE, H., 1952. The epidemiology of schistosome dermatitis ("Koganbyo") in Japan, *Tr. Roy. Soc. Trop. Med. and Hyg.* 45: 103–112.

——, and OTORI, Y., 1952. A comparison of the infectivity of *Schistosoma japonicum* occurring in Japan for *Oncomelania nosophora* and *Onceomelania formosana*, *J. Parasitol.* 38: 492.

HUTTON, R. F., 1952. Schistosome cercariae as the probable cause of seabather's eruption, *Bull. Marine Science Gulf and Caribbean* 2: 346–359.

INGRAM, A., 1924. Note on a possible intermediate host of *Schistosoma haematobium* in the Gold Coast, *Ann. Trop. Med. and Parasitol.* 18: 265–266.

JARCHO, S., AND VAN BURKALOW, A., 1952. A geographical study of "swimmers' itch" in the United States and Canada, *Geogr. Rev.* 42: 212–226.

KAGAN, I. G., 1953. Experimental infections of rhesus monkeys with *Schistosomatium douthitti* (Cort. 1914), *J. Infect. Dis.* 93: 200–206.

KEMP, S., AND GRAVELY, F. H., 1919. On the possible spread of schistosomiasis in India, *Indian J. Med. Research* 7: 251–264.

KHAW, O., 1947. An investigation on schistosomiasis, *Chinese Med. J.* 65: 129–132.

KUNTZ, R. E., 1950. Embryonic development of the excretory system in fork-tailed cercariae of the schistosomes and in a blunt-tailed brachylaemid cercaria, *Tr. Am. Micr. Soc.* 69: 1–20.

——, 1952. Natural infection of an Egyptian gerbil with *Schistosoma mansoni*, *Proc. Helm. Soc. Wash.* **19**: 123–124.

——, MALAKATIS, G. M., AND WELLS, W. H., 1954. Susceptibility of laboratory animals to infection by the Egyptian "strain" of *Schistosoma mansoni*, with emphasis on the albino mouse, 5. *Cong. Internat. Trop. Méd. et Paludisme*, Istanbul. **2**: 374–391.

——, 1955 a. Susceptibility studies in schistosomiasis. III. Infection of various experimental hosts with *Schistosoma haematobium* in Egypt, *Exptl. Parasitol.* **4**: 1–20.

——, 1955 b. Susceptibility studies in schistosomiasis. II. Susceptibility of wild mammals to infection by *Schistosoma mansoni* in Egypt, with emphasis on rodents, *Am. J. Trop. Med. and Hyg.* **4**: 75–89.

KUO, S. C., 1946. Further studies on distribution of schistosomiasis japonica in Szechwan Province, China, *J. Parasitol.* **32**: 367–368.

LANE, C., 1936. The carriage of schistosomes from man to man, with special attention to the molluscs which are their larval hosts in different parts of the earth, *Trop. Dis. Bull.* **33**: 1–15.

LEIGH, W. H., 1954. Schistosome dermatitis in a South Florida lake, *J. Parasitol.* **40**: (Suppl.) 43.

LEIPER, R. T., 1915. Report on the results of the bilharzia mission in Egypt, 1915, Part I. Transmission, *J. Roy. Army med. Corps* **25**: 1–55.

——, 1918. Report on the results of the bilharzia mission in Egypt, 1915, Part V. Adults and ova. *J. Roy. Army Med. Corps* **30**: 235–253.

LEROUX, P. L., 1948. Laboratory meetings, Royal Society of Tropical Medicine and Hygiene. Twenty-four microscope preparations and 615 camera lucida drawings, illustrating the shape and size of eggs of species of schistosomes of man and animals, *Tr. Roy. Soc. Trop. Med. and Hyg.* **41**: 428–430.

——, 1954 a. Ibid. (1) Hybridization of *Schistosoma mansoni* and *S. rodhaini*; (5) A new molluscan host (*Bulinus coulboisi*) for *Schistosoma haematobium* in Central Africa; (6) The infection of laboratory-bred specimens of *Bulinus* (*Pyrgophysa*) *forskali* from Gambia with an Egyptian strain of *Schistosoma haematobium*, *Tr. Roy. Soc. Trop. Med. and Hyg.* **48**: 3–5.

——, 1954 b. Correspondence.

LISTON, W. B., AND SOPARKAR, M. B., 1918. Bilharziosis among animals in India. The life-cycle of *Schistosomum spindalis*, *Indian J. Med. Research* **5**: 567–569.

MACHATTIE, C., AND CHADWICK, C. R., 1932. *Schistosoma bovis* and *S. matthei* in Irak with notes on the development of eggs of the *S. haematobium* pattern, *Tr. Roy. Soc. Trop. Med. and Hyg.* **26**: 147–156.

MCQUAY, R. M., JR., 1952. Susceptibility of a Louisiana species of *Tropicorbis* to infection with *Schistosoma mansoni*, *Exptl. Parasitol.* **1**: 184–188.

——, 1953. Studies on variability in the susceptibility of a North American snail, *Tropicorbis havanensis*, to infection with the Puerto Rican strain of *Schistosoma mansoni*, *Tr. Roy. Soc. Trop. Med. and Hyg.* **47**: 56–61.

MACY, R. W., AND MOORE, D. J., 1953. The relationship between *Trichobilharzia oregonensis* and *T. elvae*, etiological agents of schistosome dermatitis in the Pacific Northwest, *Science* **118**: 650.

MAGATH, T. B., AND MATHIESON, D. R., 1945. Infection of wild rats on Leyte with *Schistosoma japonicum*, *U. S. Naval Med. Bull.* **45**: 1195–1202.

——, 1946. Important factors in the epidemiology of schistosomiasis in Leyte, *Am. J. Hyg.* **43**: 152–163.

MALDONADO, J. F., AND MATIENZO, J. A., 1947. The development of *Schistosoma mansoni* in the snail intermediate host, *Australorbis glabratus*, *Puerto Rico J. Pub. Health and Trop. Med.* **22**: 331–373.

MELENEY, H. E., AND FAUST, E. C., 1923. The intermediate host of *Schistosoma japonicum* in China, *Proc. Soc. Exper. Biol. and Med.* **20**: 216–218.

——, SANDGROUND, J. H., MOORE, D. V., MOST, H., AND CARNEY, B. H., 1953. The histo-

pathology of experimental schistosomiasis. II. Bisexual infections with *S. mansoni*, *S. japonicum* and *S. haematobium*, *Am. J. Trop. Med. and Hyg.* **2:** 883–913.

MOGHE, M. A., 1945. Results of a survey on the nature and incidence of helminth infection in cattle, goats and sheep in the central provinces and Berar and Central India, *Indian J. Vet. Sc. and Animal Husb.* **15:** 222–230.

MOORE, D. V., YOLLES, T. K., AND MELENEY, H. E., 1949. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*, *J. Parasitol.* **35:** 156–170.

——, AND MELENEY, H. E., 1952 a. Adaptability of *Schistosoma mansoni* of human origin to mice and hamsters, *Exptl. Parasitol.* **1:** 157–160.

——, 1952 b. Susceptibility of rice rats (*Oryzomys palustris*) to *Schistosoma mansoni*, *J. Parasitol.* **38:** 76–79.

——, 1954. Comparative susceptibility of common laboratory animals to experimental infection with *Schistosoma haematobium*, *J. Parasitol.* **40:** 392–397.

MUKERJI, A. K., BHADURI, N. V., AND NARAIN, S., 1946. Experiments on the transmission of human schistosomiasis in India, *Indian J. Med. Research* **34:** 311–315.

NEWTON, W. L., 1952. The comparative tissue reaction of two strains of *Australorbis glabratus* to infection with *Schistosoma mansoni*, *J. Parasitol.* **38:** 362–366.

——, 1953. The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus*, *Exptl. Parasitol.* **2:** 242–257.

OLIVIER, L., 1953. Observations on the migrations of avian schistosomes in mammals previously exposed to cercariae, *J. Parasitol.* **39:** 237–246.

—— AND WEINSTEIN, P. P., 1953. Experimental schistosome dermatitis in rabbits, *J. Parasitol.* 280–291.

PENNER, L. R., 1950. *Cercaria Littorinalinae* sp. nov., a dermatitis- producing schistosome larva from the marine snail *Littorina planaxis* Philippi, *J. Parasitol.* **36:** 466–472.

——, 1953. The red-breasted merganser as a natural avian host of the causative agent of clam diggers' itch, *J. Parasitol.* **39:** (Suppl.) 20.

PINTO, C., 1944. Um ano de combate às doenças parasitárias que atacam os rodoviários da estrada Rio-Bahia, 1942 a 1943, *Mem. Inst. Oswaldo Cruz* **40:** 209–340.

——, AND DE ALMEIDA, A. F., 1945. Pentração das cercárias do "*Schistosoma mansoni*" na pele de "Canis familiaris", *Rev. Brasil. Biol.* **5:** 219–229.

PORTER, A., 1926. Notes on the structure and life-history of a variety *Schistosoma spindalis* Montgomery observed in South Africa, *South African J. Sc.* **23:** 661–666.

——, 1938. The larval trematoda found in certain South African mollusca with special reference to schistosomiasis (bilharziasis), *South African Inst. Med. Research* **8:** 1–492.

RANSFORD, O. N., 1948. Schistosomiasis in the Kota Kota District Nyasaland, *Tr. Roy. Soc. Trop. Med. and Hyg.* **41:** 617–628.

RAO, M. A. N., 1938. A comparative study of *Schistosoma spindalis* Montgomery and *Schistosoma nasalis*, n. sp., *Indian Vet. J.* **14:** 219–251.

——, 1939. A brief review of the species of schistosomes of the domesticated animals in India and their molluscan hosts, *Indian Vet. J.* **15:** 349–358.

RAPER, A. B., 1951. *Schistosoma bovis* infection in man, *East African Med. J.* **28:** 50–54.

RUIZ, J. M., 1952. Schistosomose experimental. 1. Receptividade de *Procyon cancrivorus* à infestação pelo *Schistosoma mansoni*, *Mem. Inst. Butantan* **24:** 111–113.

——, 1953. Equistsomose experimental. 3. *Cuniculus pacca pacca* e *Grison furax*, novos animais receptíveis à infestação pelo *Schistosoma mansoni*, *Mem. Inst. Butantan* **25:** 23–26.

SALOMÉ, B. Z., 1954. Schistosome dermatitis in the Netherlands, *Doc. Med. Geogr. et Trop.* **6:** 30–34.

SCHWETZ, J., 1951 a. A comparative morphological and biological study of *Schistosoma haematobium*, *S. bovis*, *S. intercalatum* Fisher, 1934, *S. mansoni* and S. *rodhaini* Brumpt, 1931, *Ann. Trop. Med. and Parasitol.* **45:** 92–98.

——, 1951 b. On vesical bilharzia in the Lango District (Uganda), *Tr. Roy. Soc. Trop. Med. and Hyg.* **44:** 501–514.

——, AND STIJNS, J. 1951. Sur la redécouverte de *Schistosoma rodhaini* Brumpt et la découverte de son hôte définitif, *Compt. Rend. Soc. Biol.* **145:** 1255–1257.

——, 1953. On a new schistosome of wild rodents found in the Belgian Congo, *Schistosoma mansoni* var. *rodentorum*, var. nov., *Ann. Trop. Med. and Parasitol.* **47**: 185–186.

——, 1954 a. On two schistosomes of wild rodents of the Belgian Congo: *Schistosoma rodhaini* Brumpt, 1931 and *Schistosoma mansoni* var. *rodentorum* Schwetz, 1953 and their relationship to *S. mansoni* in man, *Tr. Roy. Soc. Trop. Med. and Hyg.* **48**: 89–100.

——, 1954 b. Reflections on the 'Problem' of classification and nomenclature of molluscs, transmitters of schistosomes in Africa, *J. Trop. Med. and Hyg.* **48**: 125–131.

Sewell, R. B. S., 1919. The possible occurrence of *Schistosoma japonicum*, Katsurada in India, *Indian Mus. Rec.* **16**: 425–432.

——, 1922. Cercariae indicae, *Indian J. Med. Research* **10**: (Suppl.) 1–370.

Simmonds, W. L., Martin, W. E., and Wagner, E. D., 1951. Fresh-water cercarial dermatitis from Southern California, *Am. J. Trop. Med.* **31**: 611–613.

Sivalingam, V., 1949. Some Ceylon fresh-water snails and human schistosomes, *Ceylon J. Med.* **6**: 183–185.

Srivastava, H. D., and Dutt, S. C., 1951. Life history of *Schistosoma indicum* Montgomery, 1906, a common blood-fluke of Indian ungulates, (Correspondence), *Current Sc., Bangalore* **20**: 273–275.

Stijns, J., 1952. Sur les rongeurs hotes naturels de *Schistosoma rodhaini* Brumpt, *Ann. Parasitol.* **27**: 385–386.

Stirewalt, M. A., Kuntz, R. E., and Evans, A. S., 1951. The relative susceptibilities of the commonly-used laboratory mammals to infection by *Schistosoma mansoni*, *Am. J. Trop. Med.* **31**: 57–82.

Stunkard, H. W., 1946. Possible snail hosts of human schistosomes in the United States, *J. Parasitol.* **32**: 539–552.

——, and Hinchliffe, M. C., 1952. The morphology and life history of *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951, avian blood-flukes whose larvae cause "swimmer's itch" of ocean beaches, *J. Parasitol.* **38**: 248–265.

Travassos, L., 1953. Algumas observações sôbre a bionomia do *Schistosoma mansoni*, Sambon, 1907 feitas na cidade do Salvador, Bahia, *An. Acad. Brasil. Cien.* **25**: 157–165.

van den Berghe, L., 1937. A morphological study of bovine schistosomes, *J. Helminth.* **15**: 125–232.

Veglia, F., and LeRoux, P. L., 1929. On the morphology of a schistosome (*Schistosoma matthei*, sp. nov.) from the sheep in the Cape Province, *Ann. Rpt., Dir. Vet. Services, Union of South Africa* **15**: 335–346.

Vercammen-Grandjean, P. M., 1951. Sur la chaetotaxie de la larve infestante de *Schistosoma mansoni*, *Ann. Parasitol.* **26**: 412–414.

Vercruysese, R. A. F., 1953. Some remarks upon the polymorphic aspect of *Schistosoma bovis*, *Thapar Commemorat. Vol.* 289–292.

Vogel, H., 1942. Infektionsversuche an verschiedenen Bilharzia-Zwischenwirten mit einem einzelnen Mirazidium von *Bilharzia mansoni* und *B. japonica*, *Zentralbl. f. Bakteriol. und Parasitenk.* 1. Abt., Orig. 148: 29–35.

Ward, P. A., Travis, D., and Rue, R E., 1947. Experimental molluscan infection with *Schistosoma japonicum*, *Nat. Inst. Health Bull.* **189**: 95–100.

Wells, W. H., 1954. Correspondence.

Wright, F. J., and Roberts, J. L., 1944. A creeping eruption with intense eosinophilia in a case of infection by *Schistosoma mansoni*, *East African Med. J.* **21**: 282–284.

Wright, W. H., 1950. Bilharziasis as a public-health problem in the pacific, *Bull. World Health Organ.* **2**: 581–595.

Wu, K., 1938. Cattle as reservoir hosts of *Schistosoma japonicum* in China, *Am. J. Hyg.* **27**: 290–297.

Wu, L., 1953. A study of the life history of *Trichobilharzia cameroni* sp. nov. (Family schistosomatidae), *Canad. J. Zool.* **31**: 351–373.

DISCUSSION OF THE PAPER BY ROBERT E. KUNTZ

GEORGE R. LaRUE

Bureau of Animal Industry, Beltsville, Maryland

Dr. Kuntz's presentation has made it very clear that the schistosome problem is much more complex and more widespread geographically than most of us realized. Failure to recognize this complexity results in part from the lack of a comprehensive review of the species of parasites and of the hosts involved, and of the areas where schistosomes occur. Most of us have been aware of no more than certain segments of the problem. We have not realized that in one aspect or another the schistosome problem is of concern to parasitologists and workers in the health sciences in most of the inhabited parts of the globe. We owe much to Dr. Kuntz for this presentation, which certainly has suffered because of the limitation of time.

Speaking as a taxonomist, the precise identification of the species of blood flukes and their snail hosts is a matter of utmost importance. Until some of the taxonomic matters are settled there will continue to be confusion regarding the parasites and their snail hosts.

Dr. Kuntz has spoken of the difficulties in identifying the species of blood flukes, either by study of the adults, of their eggs, or of their cercariae. Many studies dealing with these stages are inadequate, sometimes for paucity of material, sometimes for differences in methods of study which may be inadequately described, and of course because of differences in the interpretation of observations. For example, one worker reports five pairs of penetration glands in the cercariae of species A, whereas another observer reports six pairs. Actually, both observations may be correct. The first worker studied only those cercariae which emerged from the snail naturally; the second observer secured his cercariae by cracking the snail. Neither worker may have considered it of importance to report on his methods of study. Moreover it is easy for the untrained or hasty worker to overlook as many as two or three pairs of penetration glands. Flame cells may be very difficult to see, and the determination of a full complement, even in the small numbers possessed by blood flukes, may require considerable skill and persistence on the part of the worker.

The cross-infection experiments reported thus far are of extreme importance in ascertaining the extent and the limitations of susceptibility of various host species. More such experiments should be conducted.

Some hybridization studies of snail hosts have yielded significant results, and additional similar studies are demanded if we are to learn the genetic background or basis of susceptibility among strains or races of snails which as yet cannot be distinguished on morphological grounds.

Let me suggest that hybridization studies between the various races of *Schistosoma mansoni*, or of *haematobium*, or *japonicum*, be carried out to test the ability of the offspring to infect various geographic races of snails or of verterbrate hosts. The hybridization of the schistosome races may be just as feasible as the hybridization of the snail hosts.

Certainly the investigation of the bionomics of schistosomes and of their host-parasite relations offers a great challenge to the efforts of many investigators.

THE NATURAL HISTORY AND CONTROL OF THE SNAILS THAT TRANSMIT THE SCHISTOSOMES OF MAN

LOUIS OLIVIER

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute,[1] *Bethesda, Maryland*

Schistosomiasis has long been recognized as a public health problem of the first magnitude. Nevertheless, it has gone essentially unchecked in most of the endemic areas. The schistosomes have not only been able to maintain themselves, but there is evidence that even now, while great strides are being made toward the control of many parasitic diseases, schistosomiasis is actually increasing in prevalence and spreading to new areas. In some areas schistosomiasis is now a more serious problem than it was some years ago. For instance, in a number of localities where schistosomiasis has long been endemic, the recent introduction of crop irrigation has been followed by increase in the prevalence of the disease (Khalil and Azim, 1935, 1938; Alcay *et al.*, 1942; and Stephenson, 1947).

In other areas there is evidence that the disease is spreading. In Brazil, a focus of the disease has appeared at Forlandia in the Amazon Basin (Machado and Martins, 1951; Sioli, 1953). Apparently, it was introduced there by migrating workers from Northeastern Brazil in relatively recent times. Foci, which appear to be new, have also been discovered recently in the south of Brazil (Coutinho and Pessoa, 1949; Ferreira and Meira, 1952; Lobo *et al.*, 1953). Apparently, the disease is still spreading in Africa (LeGac, Lemaigre, and Tournier-Lasserve, 1953). Gaud (1951, 1952) and Gaud and Jaubertie (1951) have discussed the factors involved in the possible spread of the disease in Africa and Madagascar.

In recent years interest in the disease and awareness of its great public health significance has increased greatly. Along with this has come increased interest in control and even in eradication of the disease. Efforts to control schistosomiasis are usually concerned, at least in part, with killing the snail vectors of the disease, since this offers an excellent means of breaking the schistosome life cycle. However, when snail control is attempted, it soon becomes evident that we know all too little about the natural history and control of the vectors.

The natural history of the aquatic schistosome vectors

The ensuing remarks are intended to apply only to the so-called "aquatic" vectors of *Schistosoma mansoni* and *Schistosoma haematobium*, and not to the vectors of *Schistosoma japonicum* which are amphibious.

A large proportion of the published studies dealing with the natural history of the aquatic vector snails has been concerned with their taxonomy, distribution, and efficiency as vectors. Nevertheless, it is generally agreed that the taxonomy of the snails is in a highly unsettled and unsatisfactory state. Moreover, the distribution and infectibility of the snails are also inadequately understood.

[1] Laboratory of Tropical Diseases

Other aspects of the natural history of the snails are even less well known. The bulk of the available natural history information dealing with the vectors has been published in taxonomic and epidemiological treatises, and although there are outstanding exceptions, much of the information is based on casual observations, and some is highly impressionistic.

The lack of sufficient accurate information concerning the natural history of the snails is a serious handicap to the epidemiologist and to one attempting to control the disease by interfering with transmission through the snails. We know relatively little concerning some of the most fundamental things about the vectors which might help us achieve their control such as their food habits, growth rate, life span, natural enemies, diseases, etc.

This lack of adequate information concerning the natural history of the vectors need not prevent immediate attempts at snail control, but if we are to make the best use of available means for killing snails, we must acquire more knowledge of their biology. We can learn this best by undertaking a series of long-term studies in a variety of natural habitats.

In recognition of the need for more knowledge of the vector snails, the National Institutes of Health, the Pan American Sanitary Bureau, and the Brazilian Ministry of Health organized a cooperative study of the subject in Northeastern Brazil in conjunction with field tests of promising molluscicides being made there. Some of the results of this study will be presented below. Their presentation will add a small amount to the sum of knowledge concerning the aquatic snail vectors, but the principal justification for their inclusion here is the hope that they will demonstrate the need for such information and will stimulate interest in the procurement of factual, quantitative natural history information which is essential to an adequate understanding of the epidemiology and control of schistosomiasis. The examples may also illustrate how even a relatively small amount of information gathered over a limited period of time may influence plans for the control of snails in a given area.

In Northeastern Brazil some of the snail habitats are without water throughout the annual dry season which lasts from five to seven months. The vector snails survive the dry season in these areas and reinfest the water during the wet season. This very interesting, and somewhat surprising, fact is obviously of great significance. We made a study of the manner in which both *Australorbis glabratus* and species of *Tropicorbis* survived the dry season in some of these localities. A detailed account of the results will be presented elsewhere, but the findings may be summarized as follows: it was found that there were usually very many snails present just before the water dried up after the rainy season. Soon after the water was gone living snails could be found at the soil surface in the shade and protection of vegetation and debris, but they were not normally found in exposed locations or deep in the mud. The disappearance of the snails from exposed locations was probably the result of the activities of chickens, rodents, and other scavengers that ate the unprotected snails. During the dry season, the mortality among the snails was very high and at the end of the dry season very few living snails were found. Sometimes the only survivors were very small specimens, a

few millimeters in diameter. The few snails that did survive the dry season were found principally in shaded places at or near the soil surface in debris or living and dead vegetation. These snails, because of their location, were not exposed to very high temperatures and were protected from animals that might eat them. Also, the relative humidity in the immediate vicinity of these snails was probably comparatively high.

Experiments conducted in the laboratory with the same snails showed that they do not conserve their moisture effectively at low relative humidities, and that they must be kept at high relative humidities if they are to survive long out of water. It follows, then, that the humidity of the micro-environment surrounding the snails in the field during the dry season is of the greatest importance in determining whether they survive until the return of water.

This study should be pursued further, but it is possible to use even this modest amount of information in planning more effective ways to attack the snails in the areas studied. First, since the dry season mortality is very great and the snails are at their lowest level numerically when the rainy season begins, it would seem that the best time to apply a molluscicide may be at the start of the rainy season. Also, since the estivating snails tend to stay close to the soil surface and since they require a high relative humidity, the dry season population might be reduced materially in some localities by clearing and burning the vegetation and debris. Snails not killed by this process would subsequently be exposed to lowered humidity and to the damaging effects of the sun's heat. Moreover, they would be more vulnerable to rodents and domesticated animals.

In another phase of the work done in Brazil, snails in typical habitats were observed closely over a period of almost two years in an attempt so learn their reproductive capacity and life span.

Figure 1 shows the sizes of the snails in a series of collections taken from one locality, a pool, harboring *Australorbis glabratus*, which dries completely each year and remains dry for five to seven months. A total of 22 collections were made over a period of 15 months, but some are omitted from the graph in order to simplify presentation of the data. The initial collection was made after the first rains of the 1953 season when the pool had contained water for about only five weeks. The population curve was bimodal. The group of small snails represented a new generation produced by the larger snails of the other group, which had survived the dry season. As the wet season progressed, the smaller snails grew to adult size. Very few new snails appeared in the colony later in the wet season as indicated by the lack of small snails in the collections of July to September. The November collection shows the sizes of the living snails left on the bottom of the pool when it dried. Neither very small nor very large snails were present. The April, 1954, collection was made about two weeks after the first rains of the wet season and shows the sizes of the snails that survived the dry season. The subsequent collections show the entrance of a new generation into the pool during May and June with the decline of the older generation. Most of the snails produced during the wet season died during the first months of the dry season and so only lived four to eight months. There was no evidence that any

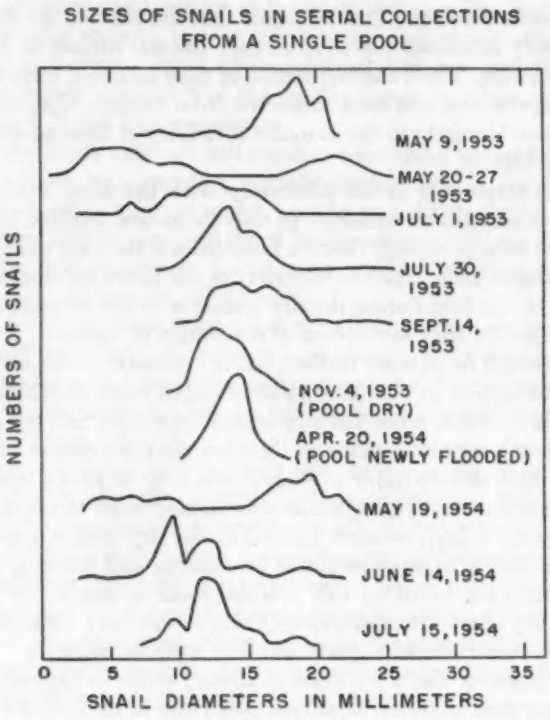


FIG. 1

snails survived two dry seasons. This and other records from snail populations living in places that dry every year show that the snails probably do not live more than 17 months, and that most of the snails live for not more than one year.

In the habitats that dry annually, transmission of the infection must be limited to the relatively short wet season. Moreover, Barbosa and Coelho (1953) have shown that infections may not be carried through the dry season in the snails, which means that during the dry season the parasite may exist only in the human host.

In the habitats that never dry there seemed to be continuous reproduction, but there were moderate fluctuations in average snail size and population density which were probably attributable to variations in reproductive activity. On several occasions it was also observed that a snail population suddenly became greatly depleted or even disappeared without apparent cause. Such a population crisis might be caused by a lack of food, a change in the natural chemical content of the water, or epidemic disease among the snails. In any event, it resulted in at least a temporary lack of schistosome vectors. The coincidence of such a "natural" population crisis and the start of snail control efforts in an area could be very misleading.

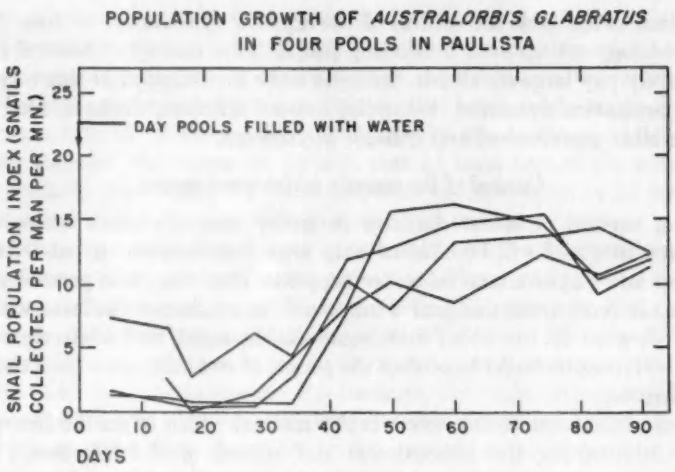


FIG. 2

A study was also made of the reproductive potential of snails that had survived the dry season in a series of typical foci. Snails were collected at frequent intervals starting at the time of the first rains of 1954 and their sizes and population densities were recorded. Population density records from four pools inhabited by *Australorbis glabratus* may be used to show the kind of data obtained (Fig. 2). (A more complete treatment of the subject will appear elsewhere.) When the first collections were made, immediately after the pools flooded early in the rainy season, the population indices were very low indicating that very few snails had survived the dry season. (An index of 1.0 means that the snails could be collected at the rate of one per man per minute.) In succeeding collections the population densities remained essentially unchanged for about four weeks while these snails grew and laid eggs, and the new generation so produced grew to collectable size (2 to 3 mm. in diameter). The population indices then increased rapidly, and about 45 days after the first collections, the indices ranged from 8.0 to 15.6 meaning that a large new generation of snails had been produced. In the following six weeks the population densities were plainly constant and the impression was gained that the saturation density had been reached in all four pools. Thus, in the space of about five weeks the snails that survived the dry season had repopulated the pools.

This enormous reproductive capacity has obvious epidemiological importance. It also means that when anti-snail measures are taken and a small number of snails are left unharmed, these snails may bring the population back to the previous level in a remarkably short time. This in turn may mean that treatment with molluscicides may have to be repeated at close intervals in some habitats if drastic reduction or extermination of the population is being attempted.

The foregoing illustrations demonstrate how even a small amount of information, collected over a relatively short period of time, may be of real help in the

formulation of methods for control of the aquatic schistosome vectors. A study of snail biology set up as a continuing project in a variety of natural habitats would surely pay large dividends. Subjects to be investigated in this way should include population dynamics, life cycle, natural enemies, diseases, food preferences, habitat preferences, and general physiology.

Control of the aquatic schistosome vectors

Lasting control of schistosomiasis in many endemic areas where aquatic vectors are involved will be effected only after improvement in environmental sanitation and improvement of water supplies. However, it is generally recognized that in most areas this goal is impossible of attainment at least within the next few decades. In the mean time, some simple, rapid, and relatively inexpensive method must be found to protect the people of endemic areas from the effects of the disease.

Control of the intermediate hosts is the method which offers the best present hope of interrupting the transmission and spread of the infection. Various methods for aquatic snail control have been proposed and some have had trial in the field. The methods include trapping the snails (Azim and Ayad, 1948; Barlow and Azim, 1945–1948), the use of fish (Cawston, 1921; Oliver-González, 1946), and ducks (Elgood and Cherry, 1919; Cawston, 1921) which feed on the snails, the propagation of snail diseases (Dias, 1953; LeRoux, 1953; Bayer, 1954), and, of course, drainage of flooded areas, stream clearance, and the control of water courses. Obviously there are many useful methods for the control of the vector snails, and a comprehensive snail control program might make use of several of the methods simultaneously. Probably the one method that best satisfies the criteria of speed, effectiveness, and reasonable economy is the use of molluscicidal chemicals.

Copper sulfate has been used against the aquatic vectors of schistosomiasis in Egypt (Barlow and Azim, 1945–1948) and other places (Annecke and Peacock, 1951; Alves and Blair, 1953), but it is not generally considered to be a highly successful molluscicide. Favorable results following use of this substance in some situations in Brazil by Bustorff Pinto *et al.* (1951) and Aguirre (1953) may stimulate interest in copper sulfate especially for use in places where fish are valued since it is less lethal for fish than most other molluscicides. Slaked lime has been used with some success against the vectors in Venezuela (Luttermoser, 1943) and Brazil (Aguirre, 1953), but it is not economical since large quantities must be used, and the labor costs are very high. Recently a search for other molluscicides has resulted in the discovery of a number of chemicals that are highly lethal for aquatic snails (McMullen and Graham, 1947; Kuntz and Stirewalt, 1950; Kuntz and Wells, 1951; McMullen *et al.*, 1951a, 1951b; McMullen, 1952; Nolan, Bond, and Mann, 1953; Bond and Nolan, 1954). Some of these substances kill snails in concentrations below the level of five parts per million and show great promise as molluscicides, but field information concerning these substances is incomplete at present. Some are unsuitable for field use for one or more reasons such as high cost, toxicity to man, inadequate solubility in water,

and so on. Nevertheless, even in the present state of our knowledge, it is possible to say that aquatic vectors living in standing water can be successfully killed by the use of several of the newer compounds at a cost that is probably not prohibitive for some of the endemic areas. On the other hand, we do not know, at the present time, how to use the available molluscicides effectively in running water. However, there is real reason to believe that at least one of the substances, sodium pentachlorophenate, will be found, after further study, to be useful and and economical in running water (Dobrovolny and Barbosa, 1953).

One of the most difficult problems in aquatic vector control stems from the fact that these vectors, at least under certain conditions and in some areas, can live for long periods out of water (Barlow, 1933, 1935; Brumpt, 1941; Annecke and Peacock, 1951; Barbosa and Dobbin, 1952; Olivier and Barbosa, 1954). The snails normally live, and must breed, in the water, but they may find themselves out of the water for various reasons. For instance, they may be removed from the water on vegetation or with the mud during stream clearance operations, or they may be left stranded when the water level is lowered due to natural or artificial causes. No doubt the capacity to survive out of water varies with the snail species, and strain differences with respect to this characteristic may exist within a species. The known molluscicides are not highly effective against dry snails living outside the water even when sprayed directly on the shells (Barlow and Azim, 1947a; Dobrovolny, 1953). Consequently molluscicidal treatment of a snail habitat containing snails living outside the water is almost sure to be incomplete. Even if all the snails in the water are killed by the treatment, the snails living outside the water may reinfest the area (Dobrovolny and Barbosa, 1953). The solution to this difficult problem is not yet at hand.

Progress in recent years toward the development of means for the control of schistosomiasis applicable in the widespread endemic areas has been very encouraging, but the problem in most of the endemic areas is still far from solution. The search for better molluscicides should continue and ways to use them efficiently in the field under a variety of conditions must be found. Search should also be made for natural enemies or diseases of the snails that might be used against them. Finally, the physiological characteristics of the snails should be studied in order to reach an understanding of the action of lethal chemicals on the snails.

With the information and materials available at present, it may be feasible in a few selected areas, to attempt eradication of schistosomiasis transmitted by so-called "aquatic" vectors. However, in most of the endemic areas, eradication is probably not attainable in the near future, and in many of the worst areas no more than a partial interference with transmission can be hoped for.

REFERENCES

Aguirre, G. H., 1953. Personal communication.

Alcay, L., Marill, F., and Musso, J., 1942. Le foyer de bilharziose urinaire de Saint-Aimé-de-la-Djidiouia (Département d'Oran, Algérie), *Arch. Inst. Pasteur, Algeria* **20**: 39–99.

Alves, W., and Blair, D. M., 1953. An experiment in the control of malaria and bilharziasis, *Trans. Roy. Soc. Trop. Med. Hyg.* **47**: 299–308.

ANNECKE, S., AND PEACOCK, P. N. B., 1951. Bilharziasis in the Transvaal, *S. African Med. J.* **25**: 676–680 and 689–692.

AZIM, M. A., AND AYAD N., 1948. A preliminary report on the value of palm-leaf traps in the survey and tre[illegible]ent of streams infested with snails, *Trans. Roy. Soc. Trop. Med. and Hyg.* **42**: 231–246.

BARBOSA, F. S., AND COELHO, M. V., 1953. Ação da dessecação sôbre as fases larvárias intra-caramujo de *Schistosoma mansoni* em *Australorbis glabratus*, *Pubs. Avulsas Inst. Aggeu Magalhães* **2**: 159–162.

BARBOSA, F. S., AND DOBBIN, J. E., 1952. Resistência de *Australorbis glabratus* à dessecação em condições naturais, *Pubs. Avulsas Inst. Aggeu Magalhães* **1**: 141–144.

BARLOW, C. H., 1933. The effect of the "winter rotation" of water upon snails involved in the spread of schistosomiasis in Egypt, 1930–1931 and 1931–1932, *Am. J. Hyg.* **17**: 724–742.

BARLOW, C. H., 1935. Further studies of the survival, after drying, of the snail hosts of the human schistosomes of Egypt, *Am. J. Hyg.* **22**: 376–391.

BARLOW, C. H., AND AZIM, M. A., 1945, 1946, 1947a, 1947b, 1948. Annual reports of the Bilharzia Snail Destruction Section, Ministry of Public Health, Medical Affairs, Cairo for 1942, 1943, 1944–5, 1945–6, and 1946–7.

BAYER, F. A. H., 1954. Larval trematodes found in some fresh-water snails: a suggested biological method of bilharzia control, *Trans. Roy. Soc. Trop. Med. and Hyg.* **48**: 414–418.

BOND, H. W., AND NOLAN, M. O., 1954. Results of laboratory screening tests of chemical compounds for molluscicidal activity. II. Compounds of mercury, *Am. J. Trop. Med. and Hyg.* **3**: 187–190.

BRUMPT, E., 1941. Observations biologiques diverses concernant *Planorbis* (*Australorbis*) *glabratus* hôte intermédiare de *Schistosoma mansoni*, *Ann. Parasitol.* **18**: 9–45.

BUSTORFF PINTO, D., ROBERT, C., AND MAIA PENIDO, H., 1951. Resultados de experiências com diversos planorbicidas no Vale do Rio Doce, *Revista Serviço Especial Saúde Pública*, Rio de Janeiro **4**: 357–370.

CAWSTON, F. G., 1921. Wild birds a cause of the spread of bilharzia infection, *J. Trop. Med. & Hyg.* **24**: 109–110.

COUTINHO, J. O., AND PESSOA, S. B., 1949. Sôbre um foco autóctone de espuistossomose mansônica em Jacarézinho (Norte do Estado do Paraná, Brasil), *Hospital, Rio de Janeiro* **35**: 531–542.

DIAS, E., 1953. *Nova possibilidade de combate aos moluscos transmissores das esquistossomoses*, Emprêsa Editora O Êco, Bambui, Minas Gerais, Brasil. pp. 1–22.

DOBROVOLNY, C. G., 1953. Personal communication.

DOBROVOLNY, C. G., AND BARBOSA, F. S., 1953. Field trials of sodium pentachlorophenate as a molluscacide in flowing waters in Brazil, *Pubs. Avulsas Inst. Aggeu Magalhaes* **2**: 121–158.

ELGOOD, B. S., AND CHERRY, T., 1919. Bilharziasis: its incidence and eradication, *Lancet* **197**: 636–637.

FERREIRA, J. M., AND MEIRA, J. A., 1952. Três casos de esquistosommose mansoni procedentes do Interior do Estado de São Paulo (Ourinhos, Palmital, e Ipaucu). Foco autóctone na cidade do Ourinhos, *Revista Paulista do Medicina* **41**: 15–18.

GAUD, J., 1951. Revue critique des travaux consacrés à bilharziose vésicale au Maroc, *Bull. Inst. Hyg. Maroc.* **11**: 69–95.

GAUD, J., 1952. Le problème des bilharzioses a Madagascar, *Mém de l'Inst. Scientifique de Madagascar*. Series A. **7**: 117–144.

GAUD, J., AND JAUBERTIE, R., 1951. Rôle des facteurs humains dans la répartition géographique des bilharzioses en Afrique, *Ann. Parasitol.* **26**: 420–439.

KHALIL, M., AND AZIM, M. A., 1935. The introduction of *Schistosoma* infection through irrigation schemes in the Aswan Area, Egypt, *J. Egypt Med. Assn.* **18**: 371–377.

KHALIL, M., AND AZIM, M. A., 1938. Further observations on the introduction of infection

with *Schistosoma haematobium* through the irrigation schemes in Aswan Province, *J. Egypt. Med. Assn.* **21**: 95–101.

KUNTZ, R. E., AND STIREWALT, M. A., 1950. Laboratory evaluation of two dinitro-phenols as molluscacides, *Proc. Helminth. Soc. Wash.* **17**: 95–102.

KUNTZ, R. E., AND WELLS, W. H., 1951. Laboratory and field evaluations of two dinitro-phenols as molluscacides for control of schistosome vectors in Egypt with emphasis on importance of temperature, *Am. J. Trop. Med.* **31**: 784–824.

LEGAC, P., LEMAIGRE, C., AND TOURNIER-LASSERVE, C., 1953. Rôle du facteur humain dans l'épidémiologie de la bilharziose vésicale en Oubangui-Chari (A.E.F.), *Bull. Soc. Path. Exot.* **46**: 685–688.

LEROUX, P. L., 1953. Metacercarial infection responsible for heavy mortality amongst fresh water molluses, intermediaries of mammalian schistosomes, in aquaria, *Trans. Roy. Soc. Trop. Med. Hyg.* **47**: 265.

LOBO, A., LUZ, E., AND CONSOLIN, J., 1953. Novos focos de esquistossomose mansônica no estado de Paraná. (In press, Proc. XI Congresso Brasileiro de Hygiene.)

LUTTERMOSER, G. W., 1943. Destrucción de caracoles transmisores de *Schistosoma mansoni* en Venezuela, *Rev. San. Asist. Social.* **8**: 3–44.

MACHADO, W. G., AND MARTINS, C., 1951. Um foco autóctono de schistosomose no Pará, *O Hospital* **39**: 289–290.

MCMULLEN, D. B., 1952. Schistosomiasis and molluscacides, *Am. J. Trop. Med. & Hyg.* **1**: 671–679.

MCMULLEN, D. B., AND GRAHAM, O. H., 1947. The control of schistosomiasis japonica. II. Studies on the control of *Oncomelania quadrasi*, the molluscan intermediate host of *Schistosoma japonicum* in the Philippine Islands, *Am. J. Hyg.* **45**: 274–293.

MCMULLEN, D. B., KOMIYAMA, S., ISHII, N., ENDO-ITABASHI, T., AND MITOMA, Y., 1951a. Results obtained in testing molluscacides in field plots containing *Oncomelania nosophora*, an intermediate host of *Schistosoma japonicum*, *Am. J. Trop. Med.* **31**: 583–591.

MCMULLEN, D. B., KOMIYAMA, S., ISHII, N., ENDO-ITABASHI, T., OZAWA, K., ASHAKAWA, T., AND MITOMA, Y., 1951b. The use of molluscacides in the control of *Oncomelania nosophora*, an intermediate host of *Schistosoma japonicum*, *Am. J. Trop. Med.* **31**: 593–604.

NOLAN, M. O., BOND, H. W., AND MANN, E. R., 1953. Results of laboratory screening tests of chemical compounds for molluscicidal activity. I. Phenols and related compounds, *Am. J. Trop. Med. Hyg.* **2**: 716–752.

OLIVER-GONZÁLEZ, J., 1946. The possible role of the guppy, *Lebistes reticulatus*, on the biological control of schistosomiasis mansoni, *Science* **104**: 605.

OLIVIER, L., AND BARBOSA, F. S., 1954. Survival and weight loss of *Australorbis glabratus* from Pernambuco, Brazil, when kept out of water at various relative humidities, *J. Parasitol.* **40** (Supplement): 36.

STEPHENSON, R. W., 1947. Bilharzia in the Gezira irrigated area of the Sudan, *Trans. Roy. Soc. Trop. Med. Hyg.* **40**: 479–494.

SIOLI, H., 1953. Schistosomiasis and limnology in the Amazon Region, *Am. J. Trop. Med. & Hyg.* **2**: 700–707.

DISCUSSION OF THE PAPER BY L. J. OLIVIER

HENRY VAN DER SCHALIE

Museum of Zoology, University of Michigan, Ann Arbor, Michigan

Before commenting on technical aspects of Dr. Olivier's paper, I would like to emphasize some points made in the Introduction. In view of our mutual experiences, two concepts need especially to be stressed: (1) that schistosomiasis is spreading rapidly; and (2) that there is an urgent need for obtaining better knowledge of the biology (in its broadest sense) of the vector snails.

With regard to the spread of schistosomiasis, three countries in another part of the world (Egypt, Sudan, and Iraq) will be mentioned. It is important to note that in Egypt more than half of the population, or an estimated 15 million people, have intestinal or vesicular schistosomiasis or both. Under present conditions of sanitation, education and medical treatment, the only hope of alleviating this serious situation lies in the control of the vector snails. It must be remembered that Egypt is overpopulated and in order to feed the growing population the country is forced to bring more and more of the Nile Valley into perennial irrigation. Barlow, Scott, Abdel-Azim, and others, have indicated that wherever the system of drainage has been changed from basin to perennial irrigation, the human incidence of schistosomiasis has increased from about 6 to 60 per cent or more. At present there are various schemes in Egypt for converting large areas to perennial production. The spread of schistosomiasis in these regions may well cancel many of the benefits envisioned by those who ignore the threatened increase of this debilitating disease.

The history of the spread of schistosomiasis in the Gezira irrigated area of the Sudan has been well documented by Greany and others. This region, with some million acres now planted to cotton and other crops, was without canals and snails a little more than 25 years ago. Both species of *Schistosoma* (*mansoni* and *haematobium*) were brought to the area and both snail vector groups (*Planorbis* and *Bulinus*) have become widely distributed. It is estimated that among the quarter million people who live in the Gezira, at least one out of five has schistosomiasis. Because of the alarming rate of spread, the Sudan Government and the World Health Organization are interested in finding better means of control. Dr. Naguib Ayad and I were privileged to observe the conditions in the Gezira during July. Our report and recommendations are contained in a WHO report to the Eastern Mediterranean Regional Office, published August 27, 1954.

In Iraq the problems of spread are somewhat similar to those already indicated for Egypt and the Sudan. When large areas of land are reclaimed by the perennial system of irrigation, which will take place when the waters of the Tigris and Euphrates are again harnessed, the incidence of schistosomiasis will undoubtedly develop to serious proportions.

All known methods of attack, such as snail control, sanitation, human treatment and health education, should be developed. Under the economic and social conditions that prevail, however, the greatest hope for success undoubtedly lies in vector control. Dr. Olivier's paper emphasizes the need for studies of the bi-

ology of these snails. As a malacologist, I will take this opportunity to make a few observations. At present there is urgent need for developing the field of medical malacology, but there are all too few schools that have the facilities and staff to offer the necessary training. My own efforts to obtain support for malacological work from existing agencies have not been encouraging. Actually some of the best studies for the mollusk vectors are being done by parasitologists. Malacologists are indebted to them for numerous contributions, such as this one by Dr. Olivier.

The portion of Dr. Olivier's paper that deals with the work done in Brazil is in essential agreement with the results obtained in a recently completed joint project of the Egyptian Government and the World Health Organization, known as "Egypt 10." This is important because neither group was aware that the other was working with the vectors under field conditions. In Brazil it was found that snails harboring schistosome larvae were unable to survive periods of hibernation. Our records for the past two years in Egypt indicate that after the winter "closure" there were no cercaria-shedding snails in the canals until about the middle of May. It is evident that snails infected prior to closure did not survive the period of hibernation. Also, the canals again became polluted as soon as the water was returned to them and the surviving snails did not shed cercariae until new infections had developed. These circumstances have historic significance in that they undoubtedly account for the difficulty experienced by Leiper and others in discovering which snails were involved in the life histories of the human schistosomes in Egypt. Dr. Olivier's observations that the hibernating snails do not burrow into the mud also agrees with ours. The animals may utilize moisture left in crevices or be covered by silt but they do not actively penetrate the soil.

In summary, I would like to stress that although many details of the biology of the vectors have yet to be discovered, it is important that we attempt to apply what is known. For example, if we consider the two significant observations briefly mentioned above, it is likely that that information may prove useful in planning a control program in Egypt. By instituting properly spaced periods of closure it may be possible to prevent infected snails living long enough to shed cercariae. Also, in a country that has an average annual rainfall of 3 inches or less, it would be worthwhile considering mechanized "clearance", i.e., removing the mud and sediment and the adhering snails in such a way that the vectors are completely removed from the canals. An experimental program designed to test the efficacy of these two techniques would be worth attempting in the field. If it were effective it would then be necessary to determine how the procedure could be utilized and integrated with current agricultural practices. It should be emphasized, however, that conditions under which vectors live, as well as the customs of the people, differ considerably in infested regions. These differences are marked when Egypt is compared to the Sudan, even though the snail fauna is essentially the same in the Nile of both countries. The need for detailed knowledge of the biology of the vectors is especially important in control programs in areas where differences occur in irrigation systems, agricultural practices, mores of the people, etc.

THE BIOLOGY AND CONTROL OF THE AMPHIBIOUS SNAILS THAT SERVE AS INTERMEDIATE HOSTS FOR *SCHISTOSOMA JAPONICUM*

LAWRENCE S. RITCHIE

406th Medical General Laboratory, APO 500, San Francisco, California

Apparent limitations of molluscicides against *Oncomelania* snails, imposed by environmental conditions, have prompted increased emphasis on fundamental studies of snail biology (McMullen, 1952; McMullen *et al.*, 1953). It is possible that derived information, when properly integrated with agricultural practices, engineering pursuits and methods of applying molluscicides, will suggest more effective control of schistosomiasis japonica.

Extensive investigations on morphology, bionomics, reproduction and control of *Oncomelania* are currently being undertaken by several agencies.[1] The nature and results of these, which are presently available only as progress reports or personal communications, will be incorporated with a general review of the literature.

BIOLOGIC STUDIES OF *ONCOMELANIA*

Morphology

Morphologic studies, such as those of Li (1934) and Abbott (1948), are being reviewed and extended at the 406 MGL as a basis for determining pathogenesis elicited by molluscicides. Reconstructions of muscular, digestive and reproductive systems are in progress using serial photographs of histologic sections. Prerequisite observations on normal tissues constitute a basis for corollary observations on snail physiology, bionomics and reproduction. The project readily encompasses observations on the schistosome cycle in the snail. In this regard it appears that the infection is localized in the gonads rather than the "liver". Gonadal tissues are essentially displaced by the parasite, warranting the inference that at least temporary sterility results (J. E. Williams, pers. comm.). This phenomenon was observed and referred to by Nagano (1927) as "parasitic castration".

[1] Yamanashi Medical Research Institute, Kofu City, Yamanashi, Japan
National Institute of Health, Tokyo, Japan
Department of Medical Zoology, Kyoto University of Medicine, Kyoto, Japan
Department of Parasitology, Kurume Medical College, Kurume, Japan
Department of Zoology, Taiwan University, Taipeh, Formosa and Department of Hygiene and Preventive Medicine. College of Medicine, University of Iowa, Iowa City, Iowa
School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, California
Bilharzia Control Project of the Philippines, Palo, Leyte, P. I.
406th Medical General Laboratory, APO 500, San Francisco, California

Ecology

General description of habitat. The primary habitat of *Oncomelania* is not the rice field, but rather the terminal or feeder irrigation ditches. The habitat of *O. quadrasi*, which is somewhat different, has been described as occurring primarily in marshlands and on wet, heavily vegetated borders of slow-flowing streams. Although the habitat of *Oncomelania* varies greatly, field observations reveal certain basic features of all. Investigators are agreed on the importance of dense vegetation and water. Vegetation makes a multiple contribution in supplying humus as food, suppressing evaporation, which is particularly important in marsh habitats, and serving as a temperature control through benefit of shade. Uninterrupted wetness, which is imperative for propagation, is very effectively supplied by irrigation, while benefits of subterranean seepage, rain and dew suffice in marshlands.

Ritchie *et al.* (1953), after surveying *O. nosophora* along the Tone River, Japan, where snails are essentially limited to broad marshlands between dikes and channel, suggested that this environment may represent the original habitat, while irrigation channels are a secondary adaptation. Similarly, McMullen *et al.* (1953), after finding *O. quadrasi* in stream origins of virgin forests, stated, "... the commonly accepted relationship between the disease, man and rice farming is a comparatively recent development."

Foods. Investigations at the School of Tropical and Preventive Medicine, Loma Linda, California (1954) have shown that snails survive longer on filter paper alone than on either of nine other substances, including fish foods, leaves, soil, liver extract, weathered wood and celotex. When filter paper is introduced into laboratory colonies, the response is spectacular. Snails soon collect on it and remain until it is consumed. A search for a special cellulose-splitting agent is indicated. Following experimental feedings, Izumi (1951) found that algae, agar, starch, common greens, grasses and fine soil had been ingested. Soil containing peptone was avoided, as were silkworm chrysalids, while soil with starch was consumed. It appears that decaying matter, high in cellulose content and poor in protein, and living unicellular organisms of the soil constitute primary foods. Whether the juvenile snails depend on a different diet than adults must also be investigated.

Water. Komiya and Yasuraoka (1953) noted that 60–75 per cent of *Oncomelania nosophora* specimens crawled out of water within 24 hours from depths of 10 and 20 cm. On reaching the water-line they wandered freely in and out of the water. Li (1953) found that about 75 per cent of *O. formosana* were above the water-line in monthly collections from April through October, while for January through March, 75 per cent of the specimens were in the water; in January and February the population was primarily adult. Snail activity appears to be characterized by a delicate amphibious balance, being neither decidedly aquatic nor terrestrial.

Cort (1919) demonstrated marked resistance of *O. nosophora* to desiccation, and Kawamoto (1953) showed that survival is greatly increased by occasional

wetting. *Oncomelania* adults in dry, cotton-stoppered tubes survived as long as 47 days in summer and 120 days in winter.[2] With brief wettings at intervals varying from 1 to 4 weeks, summer survival for adults increased from 47 to 126 days and for immature specimens from 15 to 147 days. Snails were dried superficially before being returned to the stoppered tubes. Such survival is particularly impressive since feeding was excluded throughout the test.

Uninterrupted wetness must prevail during the period of propagation. The decidedly aquatic tendency of juveniles is apparent (Mao, 1948; Li, 1953). The aquatic environment for this phase of molluscan life cycle is admirably supplied by irrigation water. Information regarding the degree to which eggs and newly-hatched snails can survive dryness is limited. Abbott (1948) found that eggs survived 12 hours drying at room conditions (84°F.) and some were alive even after 48 hours.

Observations on pH. Slight to medium acidity of the natural environment has been noted repeatedly (McMullen, 1947). Whether this is a limiting factor or merely coincidental is not certain. Ishii and Tsuda (1951) reported the occurrence of acid soils in both snail-inhabited and noninhabited areas in Japan; in fact, acid soils prevail throughout Japan. Experimental studies on pH, particularly in relation to juvenile snails, is needed.

Light. Kawamoto (1952a, b) found that both mature and immature *O. nosophora* responded negatively to strong direct light, while the response to weak light was positive. Ordinary room light elicited a positive phototropism in summer, but either negative or no response occurred in winter. In spring and fall the transition between these two reactions was evident.

Experiments in progress at the NIH, Tokyo (K. Yasuraoka, pers. comm.) have shown that snails stimulated with two light beams of varying intensity (1:1, 2:1, 4:1 and 10:1) responded positively to the stronger light. When "neutral-tinted" filters were placed before a light eliciting positive phototaxis, a "shadow reaction" was noted, as manifested by instantaneous contraction into the shell. A 10 per cent interference of light produced no response; 30 per cent reduction was sufficient to cause a reaction by 25 per cent of the specimens; 60 per cent reduction stimulated most; and all snails without exception contracted into the shell with 90 per cent light reduction. Sudden increases of light intensity did not cause any response.

Gravity. Yasuraoka (1954) noted a negative geotropism at a 7° angle of inclination, but not at 2° to 5°. As the angle was increased the reaction became correspondingly stronger. The rate of movement was not affected by the increase. Kawamoto (1953) reported evidence of a negative geotropic response at 2° with an increasingly stronger reaction at larger angles. At 5° and 7° the response to gravity could be nullified by a positive response to light, but at 10° and higher the stimulus of gravity superseded that of light.

Soils and minerals Ishii and Tsuda (1951) demonstrated for *O. nosophora* that on the basis of survival alone texture of soils per se was not critical, but

[2] The author stated only that the snails were kept at room temperatures, which for Japanese laboratories might be relatively low.

Hosaka *et al.* (1953) noted better growth on sandy and pebbly soils. The relation of mineral content of soils to snail requirements awaits investigation.

Temperature. Abbott (1948) observed that 23–30°C. was optimal for *O. quadrasi*. Of three temperatures tested at Loma Linda (1954) more young occurred at 26°C. than at 20° and 32°C. Maximum and minimum limits of tolerance have not been determined. It has been noted by Kawamoto (1952b) that loss of motility and hibernation occur at about 10°C. Cursory observations by the 406 MGL suggested that peak temperatures of the *O. nosophora* micro-environment are 30–32°C., while coincident air temperatures were as high as 38°C. Water temperatures of 40°C. were noted in paddy fields before the rice afforded shade. The latter may limit the occurrence of snails in the fields. Since water in the channels rarely exceeds 28°C., the fan-shaped distribution of snails commonly observed at the inlet may be linked with the coolness of affluent waters.

Shade, as a critical agent of temperature control, was strikingly demonstrated in the case of *O. formosana* by Hsü *et al.* (1952). This species is primarily limited to ditches shaded by bamboo brakes, which occur around dwellings and are located systematically between the fields to check winds. Irrigation systems in Japan in essence constitute a summer-long "cooling system" as well as supplying uninterrupted wetness for the environment of *O. nosophora*.

Reproduction and growth

Copulation. Where climate induces hibernation of *Oncomelania*, copulation is most common in the spring at the onset of activity (Sugiura, 1933; Hsü, 1950; Li, 1953). Li noted that 19 per cent of the *O. formosana* collected in February were mating, while for January, March and April the figures were 3, 6 and 5 per cent respectively. From May through October less than 1 per cent were paired and in November, 2 per cent. Seasonal occurrence of copulation for *O. quadrasi* has not been reported; Abbott (1946) stated only that it is most frequent during rainy or cloudy weather.

Egg-laying habits. Oncomelania eggs were initially observed by Sugiura (1933). The eggs with their clear gelatinous cover are deposited singly and covered with a characteristically molded and firmly cemented layer of mudlike material which Abbott (1946) reported as snail feces. This use of feces has been conclusively demonstrated at the 406 MGL by maintaining snails in the absence of soil, but discriminate use of feces in the presence of soil has not been demonstrated.

The preference for soil as a laying site by *O. nosophora* (1951 Ann. Rept., 406 MGL) has been confirmed at the Loma Linda laboratory (1954), where it was noted that over 90 per cent of the eggs were deposited on soil, while such materials as filter paper, gravel, brick, leaves, wood and sand were generally ignored. *O. quadrasi* showed a preference for brick as a laying site with indiscriminate selection of other objects. This tendency to select objects rather than soil makes it easier to find *O. quadrasi* under natural conditions. Thus far it has not been possible to actually demonstrate *O. nosophora* eggs in the field; apparently they are located in the soil rather than on its surface.

Whether the snails lay above or below the water-line was conjectural until

the observations at Loma Linda (1954) which showed that 95 per cent of *O. nosophora* eggs were just above the water-line, whereas in the case of *O. quadrasi* one-third were below it. Water level fluctuations reduced the number of observed *O. nosophora* young, while no reduction was apparent for *O. quadrasi*.

Periodicity of egg-laying. Ishii and Tsuda (1951) observed that laying started in May in the laboratory and ended about the first of August. This probably approximates its occurrence in nature in Japan as indicated by appearance of young in monthly snail collections (McMullen *et al.*, 1951a; and 1951 Ann. Rept., 406 MGL). It appears that seasonal periodicity is imposed by the environment, as laying has been observed in the laboratory during fall and winter months at the 406 MGL and the Loma Linda laboratory (1954). Li (1953) observed the new generation of *O. formosana* as early as February with maximum numbers in March; laying presumably extended from January through March. In the Philippines, observations on periodicity are inconclusive. Abbott (1946) reported increased laying with the onset of the rainy season (October), but McMullen (1947) observed some young at all times and at least one brood at each of four stations during the dry period of May to October. In China there is probably only one reproductive period per year as in Japan and Formosa, although two have been reported for specific localities in China by Watt, and Rose and Koh (see Hsü, 1950). A possible explanation of this exception is that large numbers of immature snails might have been present in the spring so that the two broods may have been produced by two different groups of adults.

Incubation. Incubation periods of 10–14 days have been reported (Abbott, 1946; Ishii and Tsuda, 1951). At the 406 MGL (1952 Ann. Rept.) it has been found that initial hatching occurred 11–13 days after laying, but it was further observed that the majority of eggs required longer; in fact, hatching continued through the 35th day of incubation. This range was the same whether the eggs were laid in March or in May and June. For those of the latter months, maximum hatching occurred after 18–19 days of incubation, while those laid in March showed a peak of 24–25 days. This difference was coincident with a temperature variation. Thus the continued appearance of juveniles in the population might exceed the interval of egg-laying by as much as three weeks. This staggering of hatching may constitute a safety factor against catastrophic events.

Persistence of egg-laying by O. nosophora *females after isolation from males.* At the 406 MGL (1954 Ann. Rept.) it has been found that during the first summer of isolation females produced as many young as the controls. Those isolated continuously through two summers produced offspring in greatly reduced numbers during the second, while no young occurred during the third summer.

Snail growth. O. nosophora juveniles attain a size of 4 mm. in about six weeks (Sugiura, 1933) and the rate of growth is about 0.4–0.5 mm. per week (McMullen *et al.*, 1951a, and 1950 Ann. Rept., 406 MGL). Li (1953) noted that *O. formosana* grows about 0.3 mm. per week. McMullen (1947) calculated a rate of 0.25 mm. for *O. quadrasi* which was essentially confirmed by investigators of the Bilharzia Control Project. P. I. (1954 Prog. Repts., May and June). Essentially no growth occurs for *O. nosophora* between October and April (McMullen *et al.*, 1951a;

Ritchie and Hunter, unpub.). Rates of growth at varied temperatures is under investigation at Loma Linda.

The life span. The life span of *Oncomelania* has not been conclusively established. Of 100 *O. nosophora* painted and returned to the point of collection, Sugiura (1933) recovered 63 after three years, but only 4 after the fourth year. Since they were a year old when marked, it appears that they may live as long as five years; such results have been obtained under laboratory conditions at the 406 MGL. McMullen *et al.* (1951a) did not recognize any peak of mortality during a period of two years. Contrarily, Li (1953) noted high mortalities among adults of *O. formosana* after a period of reproduction, and Kawamoto (1952b) noted that dead *O. nosophora* are most prevalent in August. It is important to determine whether there is any periodicity of mortality for *Oncomelania*.

CONTROL OF *ONCOMELANIA*

Naturalistic measures

Agricultural and related engineering practices. The original effect of rice culture on the density and distribution of *Oncomelania* is only conjectural. Inhabitable areas must have been greatly reduced, as most of the land was transformed into paddy fields which are essentially unsuitable for this genus. Such a loss, however, may have been offset by increased population density in the reduced habitat of the irrigation channels. Uninterrupted wetness and concomitant temperature control afforded by cool irrigation waters during the reproductive period may have constituted improvements of the snail environment. The localization of *Oncomelania* in irrigation channels, combined with increased activities of definitive hosts in this same area for tillage, undoubtedly increased the threat of infection for both the intermediate and definitive hosts. Thus it was, probably, that schistosomiasis became identified with rice culture. These observations are consistent with those of Faust (1952), who stated that extension of irrigation projects has increased the distribution of the disease, and that naturalistic controls must be applied to both existing and planned irrigation systems. Conversely, the delineation and concentration of snails in irrigation channels may in the end facilitate their control. If success is ultimately achieved with molluscicides, it will be predicated on this fact, and the same would be true of other measures.

An exception of this apparent adaptation to conditions of rice culture seems to have occurred in the reclaimed marshlands outside the dikes of the Tone River (Ritchie *et al.*, 1953). In such areas, drainage tends to supplant irrigation as the primary engineering problem. Application of drainage or flooding as a snail control measure is a phase of the Bilharzia Control Project in the Philippines (1954 Prog. Repts., July).

Cementing of irrigation channels is included in the current control program in Japan. Although snails persist, they are reduced in numbers (Okomoto, 1952), and it is possible that some are carried in by irrigation waters rather than being reproduced there. Too often cementing is limited to other than terminal ditches, but if projected to include the latter, other inexpensive control measures could be effectively integrated. This measure is costly, but incorporates agricultural benefits. Its merits may not be fully recognized for a quarter of a century.

Physical destruction of cercariae was suggested by Cawston (1946), who recommended "forced disturbance" of water as a means of damaging the cercariae. Sugiura and Ota (1954) tested for possible damage to cercariae by pouring them from various heights, after Sugiura (pers. comm.) had noted that boys swimming above a waterfall of two meters became infected while those swimming below did not.[3] Damage appeared to result more from the quantity of water poured than from height. When the volume was 200 ml., tails were broken from 78, 93 and 95 per cent of the cercariae at 30, 60 and 100 cm. respectively. Infections did not occur when hamsters were exposed to tail-less carcariae. Baffles and rapids as well as waterfalls might prove to be effective and economical implementations of this observation.

Certain agricultural practices, such as burning and cleaning of ditches, tend to reduce snail populations, but they are ineffective, as all farmers do not perform them in any one season. In many ditches there is enough dead vegetation, which if burned during dry periods of early spring, would account for 30-50 per cent mortalities; and, as shown by Okabe (1952), such figures could be greatly increased by adding a little straw for burning. Farmers commonly remove varying quantities of soil in the maintenance of their irrigation channels, thus reducing the number of snails (McMullen *et al.*, 1951a). The accumulative effect of these practices, if carried out simultaneously and followed with molluscicidal applications, could bring about extraordinary benefits. The importance of utilizing all benefits which could be obtained from normal agricultural pursuits, plus the integration of specific control measures must be stressed.

Biologic controls. Sugiura and colleagues (pers. comm.) have repeatedly shown that crayfish, in the absence of other *foods*, will crush and consume large numbers of *O. nosophora*. If the snails are included in its natural diet, this crustacean might prove an effective agent of control. It may, however, be a hazard to rice culture, damaging the rice plant and honeycombing the retainer walls of the paddy field. The crayfish at present are spottily distributed in Yamanashi, and it remains to be seen whether sporadic disappearance of the snails is due to their presence. This is currently under test in isolated colonies by the above investigators.

Other biologic agents which have been investigated as possible means of controlling schistosomiasis include certain fish, the Columbia ramshorn snail (*Marissa*), oligochaetes, leeches and bacteria. Nagano (pers. comm.) reported that one small carp per six square feet effectively suppressed the lymnaeid intermediate host of *Fasciola hepatica*. Observations on the effect of the *Marissa* snail on colonies of *Australorbis* in Puerto Rico have been inconclusive. Parasitic oligochaetes associated with *Australorbis* have been reported to consume miracidia as well as protozoa; destruction of laboratory colonies of *Australorbis* by leeches has been noted; and investigations have revealed a bacterium pathogenic for molluscs.

Although examples of ideal control of one biologic agent with another are few,

[3] When five rabbits were exposed at each of these places, average infections of five worms were contracted by animals above the waterfall, while none occurred below.

and the procedure hazardous, this measure should be carefully explored for the control of schistosomiasis.

Toxic agents

Laboratory screening tests. Six to eight thousand compounds have been screened by laboratory methods on the aquatic and/or amphibious intermediate hosts of schistosomes. In 1952, McMullen reported laboratory tests on about 5,800 chemicals, of which 18 were effective and 36 moderately so against *O. nosophora*. Among these the phenols predominated, including the halogenated and dinitro-alkyl phenols. Nolan and colleagues have tested about 2,000 chemicals against *Australorbis*. Among 701 phenolic compounds, Nolan *et al.* (1953) also found that the highly halogenated and dinitro-alkyl phenols were highly toxic for *Australorbis*, and added numerous metallic salts of the phenols to the effective list. They recognized structural features of toxic chemicals which might ultimately lead to the synthesis of extraordinary molluscicides. Bond and Nolan (1954) reported on 32 organic and inorganic mercuric compounds and found among them some of the most effective molluscicides yet reported. Vallejo-Freire *et al.* (1954) reported that certain quaternary ammonium compounds were as effective as sodium pentachlorophenate (PCP, Na). These and many of the metallic phenols have not been tested against *Oncomelania* snails.

Some attention has been given to synergistic and complementary effects of molluscicides. McMullen (1952) reported that combinations of a dinitro-alkylphenol with certain organic phosphates were effective in excess of the separate chemicals. Kuntz and Wells (1951) suggested possible complementary benefits of dinitro-o-cyclohexylphenol (DCHP) and $CuSO_4$. The possibilities of such increased benefits should be further investigated.

Field-trial tests. Investigations by McMullen (1952) contributed information on effectiveness of certain chemicals against *Oncomelania* under field conditions. In general the results were consistent with those obtained in the laboratory. In addition to demonstrating whether chemicals are effective under field conditions, it is essential to recognize a rate of application suitable for large field-tests. To this end the 406 MGL has carried out approximately 75 series of tests using a two-fold dilution series of five concentrations (1952 and 1953 Ann. Repts., 406 MGL).

Some of the best chemicals included:

2,4-Dinitro-6-isopropylphenol	Dinitro-o-cresol
2,4-Dinitro-6-sec-butylphenol	Pentachlorophenol
2,4-Dinitro-6-sec-amylphenol	Sodium pentachlorophenate
2,4-Dinitro-6-cyclohexylphenol	Sodium 2,4,5-trichlorophenate
2-phenyl-4,6-dinitrophenol	

Data from the field-plot dilution tests suggested that there is a well-defined threshold of *near-maximum* efficiency (snail mortalities of 90–95%) and that at least a 2- or 4-fold increase of this concentration is often necessary to approximate the MLD. The latter is a costly requirement, without assurance of eradication, while results with the former fluctuate greatly with environmental variables.

Intervening dilutions and the respective mortalities constitute critical information. Replication of tests with the same chemicals has produced variable results, indicating that the selection of a rate of application for field-tests must not be made on a single dilution series. Regardless of the difficulties encountered, the field-plot dilution test is essential in the complete evaluation of a potential molluscicide, and the two-fold, five-dilution series appears to be appropriate for obtaining necessary information.

Field-tests. Sodium pentachlorophenate (PCP, Na) and dinitro-o-cyclohexylphenol (DCHP) were first compared by McMullen *et al.* (1951b) by means of large-scale field-tests against *O. nosophora*. Application rates of about 4 to 1 respectively (0.4 and 0.1 grams per square foot, active ingredient) gave comparable results. Conversely, the cost ratio was about 1 to 4, giving a cost-efficiency ratio of approximately 1:1. This observation has been confirmed with repeated laboratory, field-plot and field tests at the 406 MGL (1952 and 1953 Ann. Repts.) and field trials by Hosaka *et al.* (1954). It is the opinion of the author that their relative merits are still not completely established.

Hunter *et al.* (1952) investigated the possible benefits of repeated applications of PCP, Na with 0.4 grams per square foot. Four applications were made to irrigation ditches of a 150-acre area in Kyushu, Japan, during the spring and fall of 1950 and 1951. A population reduction of about 99 per cent was achieved, but distinct snail foci remained. Currently, parallel field tests are being carried out by the 406 MGL (1953 Ann. Rept.) with dinitro-o-sec-butylphenol, dinitro-o-cyclohexylphenol, sodium pentachlorophenate and sodium 2,4,5-trichlorophenate. The dinitro-compounds have been applied at the rate of 0.1 gram per square foot, PCP, Na at 0.4 grams and TCP, Na at 0.2 grams per square foot. After two applications (spring of 1953 and 1954) population reductions were similar for the first three. TCP, Na was equally effective after the first application but poor results occurred at the time of the second. The latter were not conclusively the fault of the chemical.

In Japan a schistosomiasis control program subsidized by federal and prefectural funds is in progress. In Yamanashi Prefecture PCP, Na was applied in all endemic centers in 1953 and 1954. Data on reduction of snails have not been reported, but it is apparent that results have been exceedingly variable. Some funds were allocated for cementing of irrigation ditches. The Yamanashi program is undoubtedly more comprehensive than any previously undertaken against *Oncomelania*, and a publication of results will be of great interest to all concerned with schistosomiasis control.

Kuntz and Wells (1951), by means of field and parallel laboratory tests, demonstrated the effectiveness of DCHP at 3–5 p.p.m. against *Biomphalaria* and *Bulinus* snails. Repopulation varied, becoming evident after several months, though in some areas the interval was as long as one year. They found that $CuSO_4$ was more effective in running water than DCHP. Combined use of the two was suggested, as their relative merits tend to be complementary.

Extensive field-tests against *Tropicorbis* and *Australorbis* in Brazil were reported by Dobrovolny and Barbosa (1953) using PCP, Na concentrations of

5 to 60 p.p.m. with reciprocal periods of applications of 24-4 hours. Repopulation was soon apparent with less than 20 p.p.m., but at this level and above, even 4-hour applications were relatively effective. Four months after treatment, the population was still reduced at least 90 per cent in 40 per cent of all streams treated. Vaughn *et al.* (1954) also reported the successful use of PCP, Na for control of *Australorbis*. Snails were suppressed for 6 months by a single application of 15 p.p.m. in flowing water and 5 p.p.m. in still waters.

Prospectus on molluscicide evaluations. Screening of chemicals by laboratory tests have now been effectively carried out. The number of potential molluscicides thus far recognized is formidable, making it essentially impossible to apply field-trial tests to all. Expense of field work and the obvious need of replication make it imperative to introduce more extensive evaluations prior to field-trials. Potential molluscicides recognized by laboratory screening might be subjected to a battery of laboratory tests to determine not only their influence on the snail, but also the effect of environmental conditions on their action. Selections of such tests might be made from the following list:

Chemical qualities

1. Residual properties
2. Effect of temperature on toxicities
3. Effect of sunlight and organic materials on chemical stability
4. Increased effects from combinations of chemicals.

Effects of chemicals on the snail

1. Additional toxicity tests by both immersion and plate procedures for amphibious snails
2. Observations on pathological effects
3. Possible accumulation of chemical in snail tissues
4. Suppression of reproduction among snails surviving chemical applications
5. Disproportionate mortalities among infected snails
6. Effect of temperature on susceptibility of snails
7. Relative effects on snails with varying degrees of tissue water-content

Effects on other organisms as correlated with methods of application

1. Effects of chemicals on crops
2. Effects of chemicals on man and other animals

A proper sequence of tests might serve to rule out certain chemicals before the entire series was performed, and possibly only "type" chemicals should be initially tested.

By reducing the number of agents for field-trials, similar environmental conditions can be assured for a larger proportion of the total number of compounds to be tested. Replication of field-trial tests and the inclusion of molluscicides of known effectiveness as controls is imperative. A dilution series of adequate size should be used to reveal mortality patterns or deviations in mortality increments.

It is imperative that chemicals designated for large-scale field-tests be few in number. This is reflected by the fact that only a few have thus far been so tested. Certainly prior to field-tests and possibly before field-trial tests, the criterion of cost and availability should be introduced. Costly chemicals and those not available in a form suitable for application should be relegated to a hold-list. In the planning of field tests, consideration should be given to the use of several rather than a single chemical as a means of comparison; in fact, two chemicals with a very similar cost-efficiency ratio may require several years of parallel use even in large-scale control to assure fair evaluations of their respective merits.

Methods of applying molluscicides against adult Oncomelania. Molluscicides have been applied against *Oncomelania* primarily by means of the hand garden sprinkler in either the spring or fall when the irrigation ditches are dry. The effectiveness of this method has been demonstrated with two chemicals, PCP, Na and DCHP, by McMullen *et al.* (1951b) and Hunter *et al.* (1952), but the problem of carrying water as well as manning the sprinklers makes an it extremely laborious method. Success with the sprinkler has been linked with the critical supervision of the researcher, and its effectiveness will certainly decrease under less strict supervision. Application of chemical to flowing water is less laborious, but this introduces other limitations. It requires damming to raise the water level or splashing of water onto the sides and shoulders of the ditches which is effective only with relatively high concentrations. If soil is sandy, water seeps away quickly, requiring prolonged application or high concentrations; furthermore, the rate of water-flow in the irrigation systems of Japan is very rapid, ranging from 150 to 500 cubic feet per minute even in secondary ditches. These limitations necessarily result in excessive expenditure of chemical. Pressure spraying of dry ditches might prove effective and is feasible in Japan, since manual pump sprayers are a common farm item.

Eradication of *Oncomelania* even by repeated spraying appears unlikely on the basis of previous work. Their capacity to repopulate is remarkable, and is enhanced by a relatively long reproductive life-span and the fact that females can continue to reproduce for two seasons without copulation. The control project of Hunter *et al.* (1952) showed reduced repopulation the second summer of control as compared with the first and follow-up observations indicated even greater reduction the third summer, but a few snails remained.

Decreased effectiveness of repeated application is inevitable after several years, particularly when participants are volunteers, even though their own health is involved. Introduction of a less burdensome procedure after near-eradication has been achieved and repopulation is at a low level, would be highly desirable.

Application of molluscicides against juvenile O. nosophora. As a possible supplement to destruction of adult snails, both laboratory and field experiments have been carried out at the 406 MGL (1953, 1954 Ann. Repts.) to determine susceptibility of eggs and juvenile snails to molluscicides. The eggs are well protected by encasement and are probably deposited above the water-line, making them a poor point of attack; however, newly-hatched snails are essentially aquatic and remain so for about the first month of life. Laboratory tests

indicate that during the first two weeks they are much more susceptible to toxic agents than are adults. As snail reproduction is coincident with irrigation, applications would necessarily have to be made directly to the flowing water. In this regard, limitations imposed upon reproduction by seasonal variations are a distinct advantage. Information on the duration of hatching in nature, and the interval of incubation (1951 and 1952 Ann. Repts., 406 MGL) suggested that applications be made from late June through August at 2–3 week intervals. Inquiry revealed that irrigation waters could be stopped after applications for at least 24–30 hours without harm to the rice. With a six-hour application completed before midday, it was then possible to impose the added influence of peak temperatures of two days on the exposure. In 1953 six applications at 2-week intervals were made with DCHP at 1 p.p.m. with encouraging results. During 1954 only three applications at three-week intervals were made; the first was delayed by 2–3 weeks because of late rice planting. In a test area where the adult population had previously been reduced to a low level in the spring of 1954, the number of young collected during the ensuing summer was very small. In a second area when a dense population existed at onset of control (1954), the results were not as impressive. It appears that further testing of molluscicidal applications against juveniles is warranted.

Evaluations of control measures

General. Actual eradication of snails would preclude need for other evaluations, but this goal is not in sight. The incidence of the schistosome infection in the snail should be noted before and after control efforts with molluscicides, since infected snails may be more susceptible than are uninfected ones. Pre- and post-control incidence of human infection and disease are of primary importance, but methods of determination have critical limitations. The intradermal test is certainly the simplest method of determining incidence of infection, but its adequacy has not been fully evaluated. Decrease in the intensity of infection may be disproportionately greater than the decline in incidence, which would make egg-counts desirable. Whether this is feasible for *S. japonicum* is not known, but Scott (1938) stated that the output of *S. mansoni* eggs is sufficiently uniform to warrant the use of the dilution egg-count technic.

Incidence of infection in cows and dogs, as determined by a proctoscopic procedure recently introduced by Okoshi and Ishii (1952), warrants consideration; treatment of such animals as a control measure is strongly indicated for Japan.

Severity of disease has not been satisfactorily measured. An index of liver enlargement is of some value (Bang *et al.*, 1946) and decrease in the occurrence of ascites should be recorded.

Evaluation of control efforts in Japan. Concerted attempts to control *S. japonicum* in Japan have been in progress for 25 years. Although snails are abundant and the occurrence of human infections high, there is evidence that the severity of the disease has been reduced. Wright (1950) reported that considerable success has been achieved in reducing the size of the endemic area and the incidence

of infection in Hiroshima Prefecture. There is reason to believe that there has been reduction in the severity of the disease in Yamanashi Prefecture, one of the two worst endemic areas of Japan. A recent publication by the Yamanashi Prefecture (1953) includes considerable data collected over many years which indicates reduction in human incidence and commensurate reduction in the snail infection. Although ascites apparently was never very common in Yamanashi, it is now rarely seen. Added, and no less valuable assurance of reduced severity of this disease are the personal communications of devoted medical scientists. Progress was achieved with limited, though well applied, knowledge of the snail and the disease. The control measures utilized were snail control by a molluscicide, restriction against use of cattle in the fields, compulsory night soil processing, and education of the public. Currently the program in Yamanashi includes the use of a superior molluscicide, applied annually rather than intermittently over a period of years, extensive treatment of cattle and dogs, cementing of ditches, increased understanding of the public and commitments of governmental executives to deal vigorously with the problem. These are the hopeful signs of progress, which it is anticipated will be augmented by the results of world-wide research efforts.

CONCLUSIONS

Generalizations regarding measures of schistosomiasis control by recent authors might serve well as a conclusion.

Wright (1950), after careful consideration of the merits of the various measures of control, stated:

> "... the most pressing need concerns the development of a cheap, safe and effective chemical for the destruction of the snail hosts. With the availability of such a molluscicide, the problem of control would be immeasurably simplified."

Hunter *et al.* (1952), after an extensive field-test with sodium pentachlorophenate in Kyushu, Japan, stated:

> "... it is believed that the virtual eradication of schistosomiasis from Japan can be achieved at a reasonable cost with a 5–7 year program for each of the 5 endemic areas by treatment with sodium pentachlorophenate ..."

McMullen (1952, and in press), after thorough study of the snail habitat in both Japan and the Philippine Islands, stated:

> "... a control program under normal conditions should be a long enough one and take into account all the known methods of reducing the opportunity of infection. This involves problems in engineering, agriculture, disposal or treatment of definitive hosts, and sanitation as well as the use of molluscocides. Anything less than total effort is doomed to failure."
>
> "Schistosomiasis control is an extremely complicated biological problem. Every time we forget this we stub our toes. Since it has so many ramifications we need persons to work on it who have had training and/or experience in Agriculture, Biology, Education, Engineering, Epidemiology, Limnology, Malacology, Medicine and Public Health."

Faust (1952), in a critical review, stated:

> "The control of schistosomiasis is a complicated problem, much more difficult to solve than the control of malaria, urban yellow fever, epidemic typhus and the leishmaniases. . . . There are two major objectives in control of this disease, (a) development of a sense of personal and public health consciousness in the native population, which is the ultimate objective, and (b) immediate control measures which are feasible, such as providing safe water for domestic use, development of naturalistic methods for snail control in present and projected irrigation systems and the application of molluscacidal chemicals. . ."

Meleney (1954), in a very thoughtful review, stated:

> "Perhaps an ideal drug for treatment and a cheap and more highly effective molluscicide will be discovered to simplify the attack on schistosomiasis. Such efforts must be vigorously prosecuted and generously supported. Meanwhile it is important to continue basic studies of both the metabolism of the worms and the biology and ecology of the intermediate hosts so as to approach the problem more intelligently. There are few endemic areas where one method alone can accomplish control. Each area must be studied and a combination of education of people in hygiene, and specific education concerning the disease are important adjuncts to medical and public health measures."

The importance of pursuing maximum benefits from molluscicidal agents is apparent. The relative control benefits of treatment and sanitation, and feasibility of individual protective measures are conjectural, but their general merits warrant that they be integrated into large-scale control efforts. Extraordinary difficulties associated with schistosomiasis control make fundamental biologic research imperative. Naturalistic measures, including normal agricultural and engineering practices, and biologic controls should be critically investigated, as they may include the least expensive procedures. Reservoir hosts, in the case of schistosomiasis japonica, must not be neglected. In Japan the relative importance of the cow and dog is strongly indicated.

ACKNOWLEDGMENTS

The author gratefully acknowledges the assistance of Major Lyman P. Frick, 1st Lt. Arthur P. Moon and Mr. James E. Williams who gave extensive assistance in correcting the manuscript. Thanks are due Miss Marian Fitch for editorial assistance and careful preparation of the manuscript.

REFERENCES

Abbott, R. T., 1946. The egg and breeding habits of *Oncomelania quadrasi* Mlldff., the schistosomiasis snail of the Philippines, *Bull. Mus. Comp. Zoöl., Harvard Coll.* **1**: 41–48.

—— 1948. Handbook of medically important mollusks of the Orient and the western Pacific, *Bull. Mus. Comp. Zoöl., Harvard Coll.* **100**: 246–328.

Ann. Rep., 406 Med Gen Lab., 1950, 1951, 1952, 1953, 1954.

Bang, F. B., Hairston, N. G., Graham, O. H., and Ferguson, M. S., 1946. Studies on schistosomiasis japonica. II. Methods of surveying for schistosomiasis japonica, *Am. J. Hyg.* **44**: 315–323.

Bilharzia Control Project, Philippine Islands, Prog. Rep. April, May, June and July, 1954.

BOND, H. W., AND NOLAN, M. O., 1954. Results of laboratory screening tests of chemical compounds for molluscicidal activity. II. Compounds of mercury, *Am. J. Trop. Med. & Hyg.* **3**: 187–190.

CAWSTON, F. G., 1946. The control of schistosomiasis with due consideration of the destruction of larvae, *Acta Med. Scand.* **126**: 237–240.

CORT, W. W., 1919. On the resistance to desiccation of the intermediate host of *Schistosoma japonicum* Katsurada, *J. Parasitol.* **6**: 84–88.

DOBROVOLNY, C. G., AND BARBOSA, F. S., 1953. Field trials of sodium pentachlorophenate as a molluscacide in flowing waters in Brazil, *Pub. Av. Inst. Aggeu Magalhaes* **2**: 121–157.

FAUST, E. C., 1952. The control of schistosomiasis, *An. Inst. Med. Trop. Lisboa* **9**: 1037–1059.

HOSAKA, Y., IIJIMA, T., AND NAKAYAMA, S., 1953. Biological studies on *Oncomelania nosophora, Japan. J. Parasitol.* **2**: 95 (Abst.).

HOSAKA, Y., IIJIMA, T., AND SASAKI, T., 1954. Studies on molluscacide field test of Santobrite and DN-1 for *Oncomelania nosophora, Japan. J. Parasitol.* **3**: 47 (Abst.).

HSÜ, H. F., 1950. A preliminary study on the bionomics of *Oncomelania* snails, intermediate hosts of *Schistosoma japonicum*, in Kiangsu and Chekiang Provinces, China, *Am. J. Trop. Med.* **30**: 397–410.

HSÜ, H. F., LI, S. Y., WANG, C. K., FAN, P. C., AND HUANG, T. C., 1952. Studies on schistosomiasis japonica in Formosa, *Am. J. Trop. Med. & Hyg.* **1**: 287–301.

HUNTER, G. W., III, FREYTAG, R. E., RITCHIE, L. S., PAN, C., YOKAGAWA, M., AND POTTS, D. E., 1952. Studies on schistosomiasis. VI. Control of the snail host of schistosomiasis in Japan with sodium pentachlorophenate (Santobrite), *Am. J. Trop. Med. & Hyg.* **1**: 831–847.

ISHII, N., AND TSUDA, E., 1951. Possibility on the spreading of *Onchomelania nosophora*, the intermediate snail host of *Schistosoma japonicum*, in other areas besides its own habitats, *Yokohoma Med. Bull.* **2**: 366–375.

IZUMI, S., 1951. Studies on food habit and inhabitable ground of *Katayama nosophora* Robson, the intermediate host for *Schistosoma japonicum, Japan. Med. J.* **4**: 125–129.

KAWAMOTO, S., 1952a. On the photophobotaxis of *Oncomelania nosphora, Med. & Biol.* **23**: 76–79.

—— 1952b. Ecological studies of *Oncomelania nosphora*. II. Growth of the snail; field observations, *Med. & Biol.* **24**: 251–253.

—— 1953. The geotropism and the survival in dried condition of *Oncomelania nosophora, Japan. J. Parasitol.* **2**: 95 (Abst.).

KOMIYA, Y., AND YASURAOKA, K., 1953. The behavior of *Oncomelania nosophora*, the first intermediate host of *Schistosoma japonicum*, in water, *Japan. J. Med. Sci. & Biol.* **6**: 451–461.

KUNTZ, R. E., AND WELLS, W. H., 1951. Laboratory and field evaluations of two dinitrophenols as molluscacides for control of schistosome vectors in Egypt with emphasis on importance of temperature, *Am. J. Trop. Med.* **31**: 784–824.

LI, F., 1934. Anatomie, Entwicklungsgeschichte, Oecologie und Rassenbestimmung von *Oncomelania*, des Zwischenwirtes von *Schistosoma japonicum* (Katsurada 1904) in China, *Tr. Sci. Soc. China* **8**: 104–145.

LI, S. Y., 1953. Studies on schistosomiasis japonica in Formosa. III. The bionomics of *Oncomelania formosana*, a molluscan intermediate host of *Schistosoma japonicum, Am. J. Hyg.* **57**: 30–45.

LOMA LINDA, see School of Trop. and Prev. Med.

MAO, C. P., 1948. A review of the epidemiology of schistosomiasis japonica in China, *Am. J. Trop. Med.* **28**: 659–672.

MCMULLEN, D. B., 1947. The control of schistosomiasis japonica. I. Observations on the habits, ecology and life cycle of *Oncomelania quadrasi*, the molluscan intermediate host of *Schistosoma japonicum* in the Philippine Islands, *Am. J. Hyg.* **45**: 259–273.

—— 1952. Schistosomiasis and molluscacides, *Am. J. Trop. Med. & Hyg.* **1**: 671–679.

—— (In press). Some of the problems in schistosomiasis control, *Proc. 8 Pacific Sci. Cong.*

McMULLEN, D. B., KOMIYAMA, S., AND ENDO-ITABASHI, T., 1951a. Observations on the habits, ecology and life cycle of *Oncomelania nosophora*, the molluscan intermediate host of *Schistosoma japonicum* in Japan, *Am. J. Hyg.* **54**: 402–415.

McMULLEN, D. B., KOMIYAMA, S., ISHII, N., ENDO-ITABASHI, T., OZAWA, K., ASAKAWA, T., AND MITOMA, Y., 1951b. The use of molluscacides in the control of *Oncomelania nosophora*, an intermediate host of *Schistosoma japonicum*, *Am. J. Trop. Med.* **31**: 593–604.

McMULLEN, D. B., HUBENDICK, B., PESIGAN, T. P., AND BIERSTEIN, P., 1953. Observations made by the World Health Organization schistosomiasis team in the Philippines, *J. Parasitol.* **39** (*Suppl*): 17 (Abst.).

MELENEY, H. E., 1954. Problems in the control of schistosomiasis, *Am. J. Trop. Med. & Hyg.* **3**: 209–218.

NAGANO, K., 1927. Studies on prophylaxis of clonorchiasis, *Tr. 7 Cong., Far East. Ass. Trop. Med.* **3**: 211–215.

NOLAN, M. O., BOND, H. W., AND MANN, E. R., 1953. Results of laboratory screening tests of chemical compounds for molluscicidal activity. I. Phenols and related compounds, *Am. J. Trop. Med. & Hyg.* **2**: 716–752.

OKABE, K., 1952. Studies on the control of schistosomiasis japonica. I. Burning *Oncomelania* with straw, *J. Kurume Med. Soc.* **15**: 31–32.

OKAMOTO, T., 1952. Studies on eradication of *Schistosoma japonicum*, *Japan. Soc. Parasitol.*, Rep. of 21 Ann. Meeting: 85–86 (Abst.).

OKOSHI, S., AND ISHII, T., 1952. Diagnosis of schistosomiasis japonica by a method of scratching the mucosa, *Japan. Soc. Parasitol.*, Rep. of 21 Ann. Meetings: 78–79 (Abst.).

RITCHIE, L. S., HUNTER, G. W. III, NAGANO, K., AND PAN, C., 1953. The distribution of the snail *Oncomelania nosophora*, intermediate host of *Schistosoma japonicum*, along the Tone River, Japan, *Am. J. Trop. Med. & Hyg.* **2**: 915–925.

School of Trop. and Prev. Med., Coll. Med. Evang., Loma Linda, Calif., Prog. Rep. I–VI and Ann. Prog. Rep. 1953–1954.

SCOTT, J. A., 1938. The regularity of egg output of helminth infections, with special reference to *Schistosoma mansoni*, *Am. J. Hyg.* **27**: 155–175.

SUGIURA, S., 1933. Studies on biology of *Oncomelania nosophora* (Robson), an intermediate host of *Schistosomum japonicum*, *Mit. Path. Inst. Med. Fakul. Niigata, Japan*, **31**: 1–18.

SUGIURA, S., AND OTA, S., 1954. On the kill of schistosome cercariae, *Japan. J. Parasitol.* **3**: 48–49 (Abst.).

VALLEJO-FREIRE, A., RIBEIRO, O. F., AND RIBEIRO, I. F., 1954. Quaternary ammonium compounds as molluscacides, *Sci.* **119**: (3093) 470–472.

VAUGHN, C. M., OLIVIER, L., HENDRICKS, J. R., AND MACKIE, T. T., 1954. Mollusciciding operations in an endemic area of schistosomiasis in the Dominican Republic, *Am. J. Trop. Med. & Hyg.* **3**: 518–528.

WRIGHT, W. H., 1950. Bilharziasis as a public-health problem in the Pacific, *Bull. World Health Organ.* **2**: 581–595.

Yamanashi Prefecture, 1953. A general review of schistosomiasis japonica in Yamanashi Prefecture, 109 pp.

YASUDAOKA, K., 1954. Geotactic creeping of *Oncomelania nosophora*, *Japan. J. Parasitol.* **3**: 46 (Abst.).

DISCUSSION OF THE PAPER BY LAWRENCE S. RITCHIE

H. F. HSÜ

Department of Hygiene and Preventive Medicine, State University of Iowa, Iowa City, Iowa

Dr. Ritchie is to be congratulated for his paper on the biology and control of the oncomelanian snails. We, the Oriental people, are especially thankful for his own painstaking studies on this problem in Japan. In the Orient there are about 33 million persons suffering from schistosomiasis japonica. It is, therefore, one of the major medical problems in that part of the world.

His review of the subject is so complete that it is difficult to add anything to it. For some time, however, the distribution of the snail vectors has puzzled me. As you know, in Japan the distribution of *O. nosophora* is confined to only five small areas. When I visited these areas I was impressed by the fact that snails were further restricted to small, isolated habitats. For many years, although the Japanese have not succeeded in eliminating the vectors in an endemic area, the mollusks have not spread into adjacent areas.

When I was working in the endemic areas on the Chinese mainland and Formosa, this same type of distribution of the local vectors was observed. Often the physical and chemical properties of the water and soil, the light, humidity, and vegetation in the uninhabited ditches, were similar or identical to those in the vicinity that supported thriving colonies. It would be of interest to find out what factor or factors influence that distribution of these snail vectors. It is possible that this knowledge would be of value in snail control.

IMMUNOLOGICAL ASPECTS OF INFECTIONS WITH *SCHISTOSOMA MANSONI*

JOSÉ OLIVER-GONZÁLEZ[1], PRESTON M. BAUMAN[2] AND A. S. BENENSON[3]

Tropical Research Medical Laboratory, U. S. Army, and the Department of Microbiology, School of Medicine-School of Tropical Medicine, San Juan, Puerto Rico

Serological studies on schistosomiasis have shown that antibodies are produced by *Schistosoma haematobium*, *Schistosoma japonicum* and *Schistosoma mansoni* (see review by Fairley, 1951). Since the mammalian host comes in contact with the cercaria, adult and egg stages of these parasites there are numerous possibilities for investigation. It appeared to the present authors that some of these had not been studied. Most of the effort has been directed toward the investigation of the specific reactions elicited in the host by these three stages of the parasite. It was hoped that they might give information that would be of value in serodiagnosis, lead to a better understanding of the pathogenesis and possibly be of use in following the effectiveness of therapy.

MATERIALS AND METHODS

Techniques for immunological tests. The methods for obtaining schistosome adults, cercariae, and eggs have been described elsewhere (Oliver-González *et al.*, 1954).

1. Preparation of test antigens. A one per cent suspension of the well pulverized powder made from each of the three stages of *S. mansoni* was made in physiological salt solution, incubated at 37°C. for 1 hour, and then left overnight at room temperature. The following morning the mixture was centrifuged at 4,000 r.p.m. for 10 minutes and the supernatant fluid removed to be used as the 1:100 stock dilution.

2. Precipitin tests. Precipitin tests against adult, cercarial and egg test antigens were performed in microprecipitin tubes, overlaying the antigen dilutions on an equal volume of undiluted sera, followed by incubation at 37°C. for 2 hours. Serial dilutions of test antigens ranged from 1:400 through 1:6.400. A positive reaction was indicated by the formation of a precipitate at the interphase, which was read arbitrarily as 4+, 3+, 2+ and +.

3. Cercarial agglutination tests. These were performed following the technique described by Liu Ch'ien and Bang (1950), which was modified in that 0.1 cc. of serially diluted sera in physiological saline was utilized, and the sera saline mixtures placed in 10 x 75 mm. test tubes. The cercarial suspensions in tubes were examined under the stereoscope at a magnification of × 20.

4. Circumoval precipitins. Eggs of *S. haematobium*, *S. japonicum* and *S. man-*

[1] Present address: Department of Microbiology, School of Medicine School of Tropical Medicine, San Juan, P. R.

[2] Department of Medical Zoology, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

[3] Camp Detrick, Md.

soni were isolated from livers or intestines of experimentally infected mice and hamsters. In performing the tests these eggs were incubated in the sera at 37°C. The details of the technique and results obtained have been described elsewhere (Oliver-González, 1954).

5. Intradermal tests. Antigen dilutions were prepared from the stock solutions and used after adequate sterilization. Five hundredths of a cc. was injected intradermally on the forearm by means of a 0.25 ml. or 1 cc. syringe. A positive reaction was considered to be one in which the sum of the diameters of the wheal at the 15 minutes reading was 4 mm. or greater than the sum of the diameter of the wheal made immediately after injection.

Antibody studies

1. In sera from individuals infected with *S. mansoni*. The individuals infected with *S. mansoni* from whom sera were obtained may be divided into the following categories: a) children aged 10–15 years with short term infections, where the tests were made 40–240 days after exposure to cercariae; b) adult individuals with long term untreated infections probably acquired during childhood and thus of many years duration; c) adults also with long term infections, but who had received Fuadin at varying periods of time. The infection had been proven repeatedly in all of these persons by fecal examination. The human sera were then tested for cercarial precipitins and agglutinins, and for adult and circumoval precipitins.

2. In sera from artificially immunized rabbits. Rabbits weighing from 2.4 to 3.4 kilos were injected with lyophilized adults, cercariae and eggs from *S. mansoni*. The lyophilized antigenic material was suspended in Freund's adjuvant and injected subcutaneously or intramuscularly. Injections consisted of 0.83 to 1.66 mg. on three consecutive days, weekly, for a period of three weeks. The animals were exsanguinated on the 12th day after the last injection. The sera were tested for precipitins against adult, cercarial and egg test antigens, for cercarial agglutinins and for circumoval precipitins.

Also attempts were made to absorb antibodies by treating the sera with either homologous or heterologous stage antigens. Five mg. of the well dried and pulverized parasitic material were added to 0.5 cc. of the sera, incubated at 37°C. for 1 hour with frequent shakings and kept at 6°C. overnight. The following morning the suspension was centrifuged at 4,000 r.p.m. for 10 minutes, the supernatant fluid used as the absorbed sera.

The non-absorbed and absorbed sera were tested for precipitins, cercarial agglutinins and circumoval precipitins against homologous and heterologous test antigens.

3. In sera from animals with single sex infections. Sera were obtained from mice exposed to cercariae coming from snails previously known to produce single sex infections. The mice were bled not earlier than 65 days after exposure. The sera from individual mice were tested for cercarial agglutinins and circumoval precipitins. Sera from mice with male and female infections were also used as controls.

4. Species-specificity of the circumoval precipitin. The sera from human

TABLE 1

Antibodies in serums from humans infected with Schistosoma mansoni

Antigen	Antibody	Serums from recently infected individuals (40 to 240 days after exposure)			Serum from individuals with long term infections (about 10 to 15 years after exposure)		
		Number of individuals tested	Number of positives, per cent	Titer range	Number of individuals tested	Number of positives, per cent	Titer range
Adult extract	Precipitin	7	3– 42.8	1:400	49	10– 24.1	1:400 to 1:1,600
Cercarial extract	Precipitin	7	7–100	1:3200 to 1:6400	49	9– 18.8	1:400 to 1:800
Living cercariae	Agglutinin	7	7–100	1:64 to 1:128	49	3– 6.1	1:2 to 1:8
Living eggs	Circumoval	7	7–100	29.4% to 53.2% eggs affected	49	49–100	49.5% to 72·8% eggs affected

subjects and from mice and hamsters infected wi h *S. haematobium*, *S. japonicum* and *S. mansoni* were tested for circumoval precipitins against the homologous as well as the heterologous egg species. Eggs from a single species or various combinations of mixtures were used in these tests.

5. Skin sensitivity to adult, cercarial and egg antigens before and after Fuadin therapy. Skin tests using adult, cercarial and egg antigens were performed on a group of 54 adult individuals infected with *S. mansoni*, who had not received any drug treatment previous to the tests. To 18 of these, Fuadin therapy was then administered and skin tests were continued at regular intervals after treatment, each time to only 5 individuals from the group of 18.

Skin tests were also performed on another group of 68 adults who had previously received Fuadin treatment. Of these 33 had received therapy within a year and in the remaining 35 it had been given from one to several years previous to the time of study.

RESULTS

Antibodies in the sera from infected humans. In the seven children studied with short-term infections the sera from only 3 showed the presence of adult precipitins (Table 1). However, high titers for cercarial precipitins and agglutinins were obtained in all of them. Circumoval precipitins also were observed in all of these cases but the number of eggs affected was rather low.

Results obtained in the study of the sera from the 49 individuals with long-standing infections differed from those with the more recent infections, both in the number of positives for the various antibodies as well as antibody titers. For example only 18.8 per cent of these cases had cercarial precipitins and 6.1 per cent had agglutinins. These positive sera also had much lower titers than those from the children with recent infections. On the other hand, the sera from patients with old infections contained a larger concentration of circumoval

precipitins, 49.5 to 72.8 per cent as compared to 29.4 to 53.2 per cent of the eggs showing a precipitate.

As a result of Fuadin therapy the circumoval precipitin content of sera diminished, causing less marked "precipitation" on the eggs and affecting a smaller per cent of eggs than those from persons who had not received treatment. This effect was most noticeable when sera were tested several months after treatment was terminated and in those individuals where treatment was effective, as determined by repeated negative stools. (See Chart 1).

Stage specificity of antibodies produced in artificially immunized rabbits. The precipitins produced in rabbits injected with powdered adults, cercariae or eggs of *S. mansoni* gave higher titers when tested against the homologous than when tested against the heterologous antigens (Table 2). Cercarial agglutinin titers were also higher in the sera from rabbits immunized with cercariae but low

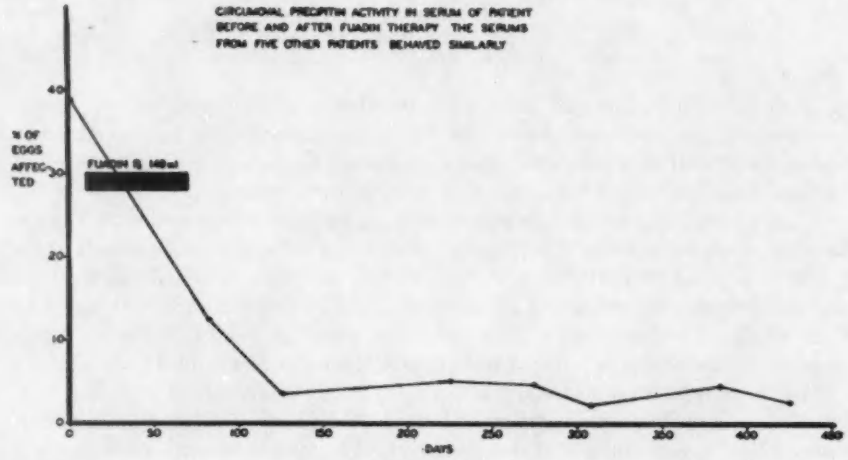


CHART 1

titers were observed in the sera from rabbits immunized with adults and eggs. When the circumoval test was used on these sera, the anti-adult sera caused no reaction about the schistosome eggs; the anti-cercarial gave only a slight reaction (3.5 to 8.4 per cent); while the anti-egg sera exhibited marked circumoval precipitation (40.9 to 43.2 per cent—Table 2).

Treatment of each antiserum with the homologous parasitic stage material always resulted in the absorption of the homologous antibody. Treatment of anti-adult sera with cercarial powder did not absorb adult precipitins. Similarly adult powder did not absorb either cercarial precipitins or agglutinins, and cercarial or adult powder did not absorb circumoval precipitins. Circumoval precipitins were absorbed only by homologous egg antigen (Table 3). This is in accord with previous work in which repeated treatment of sera with cercarial and adult worm materials resulted in no absorption of the circumoval precipitin

TABLE 2

Precipitin and agglutinin titers and in vitro *effect on eggs, of serums from rabbits artificially immunized with lyophilized adults, cercariae, and eggs**

Test Antigen	Anti-adult serum			Anti-cercarial serum			Anti-egg serum		
	Precipitins	Agglutinins	Circumoval precipitins % eggs affected	Precipitins	Agglutinins	Circumoval precipitins, % eggs affected	Precipitins	Agglutinins	Circumoval precipitins % eggs affected
Adult extract	3,200	—	—	400	—	—	0	—	—
	3,200	—	—	400	—	—	0	—	—
Cercarial extract	400	—	—	1,600	—	—	800	—	—
	400	—	—	1,400	—	—	800	—	—
Live cercariae	—	Neg.	—	—	128	—	—	8	—
	—	4	—	—	32	—	—	32	—
Live eggs	—	—	Negative	—	—	3.5	—	—	40.9
	—	—	Negative	—	—	8.4	—	—	43.2

* Figures represent values for two animals.

TABLE 3

*Antibody titers against homologous antigens in serums from rabbits artificially immunized with lyophilized adults, cercariae and eggs, before and after absorption with homologous and heterologous parasitic worm material**

Material used for absorption	Anti-adult serum		Anti-cercarial serum				Anti-egg serum	
	Adult precipitins		Cercarial precipitins		Cercarial agglutinins		Circumoval precipitin % eggs affected	
	Before absorption	After absorption	Before absorption	After absorption	Before absorption	After absorption	Before absorption	After absorption
Adult powder	3,200	Negative	6,400	3,200	128	64	48.6	47.7
	3,200	Negative	6,400	3,200	128	64	44.1	40.1
Cercarial powder	3,200	3,200	6,400	Negative	128	Negative	48.6	39.9
	3,200	1,600	3,200	Negative	128	Negative	44.1	40.3
Egg powder	3,200	3,200	6,400	Negative	128	Negative	48.6	1.3
	3,200	3,200	3,200	Negative	128	Negative	44.1	0.9
Live eggs	—	—	6,400	Negative	128	Negative	48.6	2.7
			3,200	Negative	128	Negative	44.1	1.1
Live cercariae	—	—	—	—	128	Negative	48.6	40.1
					128	Negative	44.1	37.6

* Figures represent values for two animals.

(Oliver-González *et al.*, 1954). In contrast to the above, the cercarial antibodies were completely absorbed by powdered or living eggs. This was the only instance in which there was complete absorption of antibodies against one stage by another.

Absence of circumoval precipitins in the sera from mice with single sex infections. The sera from all mice with single-sex infections were negative when tested for

TABLE 4

Cercarial agglutinins and circumoval precipitins in serums from mice with single sex-infections

Number of mice	Sex of infection	Age of infection	Cercarial agglutinins, titer range	Circumoval precipitin % eggs affected
6	Male	65 Days	1:2 to 1:4	Negative
5	Female	70 Days	1:4 to 1:16	Negative
3	Male	90 Days	1:8 to 1:32	Negative
2	Female	90 Days	1:16 to 1:32	Negative
5	Female	95 Days	1:8 to 1:32	Negative
4	Male and female	65 Days	1:16 to 1:32	37.3% to 51.5%

TABLE 5

Intradermal reactions to adult, cercarial and egg test antigens before and after fuadin therapy

Group description	Number of individuals tested	Positive reactors					
		Adult antigen 1:5,000		Cercarial antigen		Egg antigen 1:10,000	
		Number	%	Number	%	Number	%
No therapy	36	31	86.1	33	91.7	3	8.3
No therapy, therapy commenced immediately after initial skin testing	18	18	100	18	100	0	0
Therapy administered within a year	33	32	96.9	33	100	9	27.3
Therapy administered from over one to many years previous	38	37	97.4	33	94.3	25	71.4

circumoval precipitins. They were positive, however, for cercarial agglutinins. The sera from 4 mice with male and female infections, used as controls, were positive for circumoval precipitins (Table 4).

Intradermal response to adult, cercarial and egg antigens before and after Fuadin therapy. Of 54 individuals who had not received Fuadin therapy, only 3 (5.5%) gave positive reactions when the egg antigen was used in the intradermal test (Table 5). Ten patients tested 15 and 30 days after treatment were negative (Table 6). Out of 33 patients that were tested between 30 days and 1 year after treatment, 27.3 per cent were positive. In the group of 38 persons tested more than one year after treatment, 27.3 per cent were positive. In the group of 38 persons tested more than one year after treatment, 71.4 per cent were positive. It is apparent that in the treated cases the number of patients giving positive reactions with this test increased with the interval since past treatment. Intradermal tests with adult and cercarial antigens in these same individuals did not show significant differences between the treated and untreated groups (Tables 5 and 6). Similar results have been obtained in previous investigations (Oliver-González *et al.*, 1954).

Species-specificity of the circumoval precipitins. No reaction was observed

TABLE 6

Skin reactivity to adult, cercarial and egg antigens in individuals infected with Schistosoma mansoni *before and after short time intervals following fuadin therapy*

Total number of individuals in groups	Number of individuals tested	Cc of fuadin administered	Days after therapy started	Number of positives skin reactions—%		
				Adult antigen 1:5,000	Cercarial antigen 1:10,000	Egg antigen 1:10,000
18	18	None	—	18–100	18–100	0–0
	5	60 to 70	15	5–100	5–100	0–0
	5	90 to 100	30	5–100	5–100	0–0

about eggs of *S. japonicum* and *S. haematobium* when incubated in the sera from individuals or animals infected with *S. mansoni*. There was only a slight precipitation when eggs of *S. mansoni* were incubated in the human and animal serums having infections with the other two species. Marked precipitation was observed, however, when each species of eggs was incubated in the homologous antiserum. These results are in accord with those reported previously (Oliver-González *et al.*, 1954).

DISCUSSION

The results obtained above and by other investigators (Vogel *et al.*, 1948; Papirmeister *et al.*, 1948; Lui Ch'ien *et al.*, 1950) indicate a marked difference in the presence and concentrations of antibodies during short and long term infections. In early infections cercarial antibodies (precipitins and agglutinins) are easily detectable and react at high titers. It appears that these antibodies would be most useful in the detection of early cases. The titers indicate, however, that these antibodies decrease in concentration or disappear as the infection progresses. After adult precipitins became detectable there was little change during the period of infection, but the per cent showing positive reactions was small and titers were low.

The circumoval precipitin reaction, in contrast with anticercarial tests, was more effective in the older infections than in those recently acquired. This is in accord with the pattern of infection, since as the infection ages, the host receives additional antigenic stimulus from eggs being laid. The facility by which the circumoval precipitin can be detected in sera makes the test of great diagnostic value. Furthermore the fact that the antibody has a more marked effect on the eggs of the homologous than on those of the heterologous schistosome species adds more significance to the test.

The present study indicates that the circumoval precipitation concentrations decrease after the patient has received treatment with Fuadin. The reaction of sera on eggs is very slight or lacking when these are tested a year or more after effective therapy. This suggests that the test may be of value in determining the efficacy of therapy, as determined by the disappearance of anti-ovum antibodies from the patient's sera. To be of real value, however, it will be neces-

sary to determine the precise time when the titer for these antibodies decreases after adequate therapy, and what happens in the case of relapses.

The stage specificity manifested by antibodies produced in the artificially immunized rabbit is also a finding of interest. Although the adults, cercariae and eggs must have a great number of antigens in common, it appears that after the injection of lyophilized material into rabbits, the antibodies which are detectable at first are the stage-specific ones. It is to be expected, however, that if immunization had been continued the concentration of antibodies against other antigens would have increased and therefore more cross reactivity would have been observed.

Further proof of stage specificity can be seen readily in results obtained in the sera from single-sex infected animals. No circumoval precipitins were detected in single infections of females or males. Cercarial agglutinins, however, were present in the sera of these mice indicating that the infection had matured long enough for the development of antibodies. Absence of circumoval precipitins is consistent with the findings in sera from immunized rabbits where the anti-adult sera caused no precipitation about eggs. In contrast to the sera from mice with single-sex infections, sera from those with double-sex infections, used as controls, had circumoval activity.

At the time when the antiserum had the greatest activity against the homologous stage antigen, absorption of the antibody could not be accomplished by using the heterologous antigen, except when the cercarial antibodies were absorbed with egg. This suggests the presence of large amounts of cercarial antigenic factor in the egg (Table 7). Cercarial and egg antibodies appear concomitantly in the sera from monkeys 40 to 50 days after exposure to the infective organism (Bauer *et al.*, 1954). The fact that the cercarial stage as such, in the definitive host, is a transitional one, makes it seem possible that cercarial antibodies develop mainly as a result of immunization against the cercarial factor in the egg. This also may explain, in part, the persistence for many years of skin reactivity to cercarial antigens. This does not rule out, however, the possibility that after prolonged immunization, other secondary cercarial antigens in the adult may also be a factor.

The results obtained in experiments with chronic, untreated schistosomiasis, testing for circumoval precipitins, indicate that the high level antibody which

TABLE 7

Immunological relationship between cercariae and eggs

Antigen used for absorption	Antibody titer					
	Cercarial agglutinins	Cercarial precipitins	Circumoval precipitins % eggs affected	Cercarial agglutinins	Cercarial precipitins	Circumoval precipitins, % eggs affected
None	64	6,400	8.4	32	3,200	40.9
Lyophilized cercariae	Negative	Negative	10.1	Negative	Negative	39.6
Lyophilized eggs	Negative	Negative	Negative	Negative	Negative	Negative

persists can be detected easily by a simple laboratory procedure. There is also the advantage that the test can be used to determine the species of schistosome involved. In treated schistosomiasis, the circumoval antibody content of sera diminishes; therefore the test may be used to determine the effectiveness of drug therapy. The cercarial precipitins and agglutinins, although of value in diagnosis of early infections, are either negative or low titered and provide no reliable diagnosis of late infections. Complement fixing antibodies are also easily detectable in chronic untreated schistosomiasis (Fairley, 1951), but this investigation did not include an analysis of this test.

Studies on the dermal response to egg antigen have revealed certain facts not observed previously when adult or cercarial antigens were utilized. The lack of reaction obtained in intradermal tests with egg antigen during the active, untreated phase of the infection, and the positivity after therapy are interesting phenomena (Oliver-González *et al.*, 1954). This change should be investigated as a possible means of evaluating the results of chemotherapy.

SUMMARY

1. Antibody studies in the sera from human subjects infected with *Schistosoma mansoni* have revealed the presence of cercarial precipitins, cercarial agglutinins, and circumoval precipitins. Adult precipitins were also detected, but in low titers and in a small per cent of the sera tested.

2. There were differences observed in the concentration of these antibodies as related to the duration of infection. In early infections (40 to 240 days after exposure to cercariae) cercarial precipitins and agglutinins have high titers but become low or negative in infections of long duration (10 years and over). Circumoval precipitins are present in both recent and old infections but become more active in older infections.

3. The adult, cercarial, and egg antibodies as produced by artificial immunization of the rabbit are stage specific. Treatment of rabbit anti-serum with lyophilized adult or cercarial material results in the absorption of the homologous but not of the heterologous antibodies. Treatment of anti-adult, anti-cercarial, and anti-egg sera with egg material absorbs only the homologous and the cercarial antibodies. This postulates a cercarial factor in the egg.

4. No circumoval precipitins were detected in the sera from mice with single sex infections. This provides further evidence confirming stage specificity of the circumoval antibody which only develops in the presence of eggs.

5. Successful Fuadin therapy as determined by repeated negative stools and rectal biopsy reduces the circumoval activity of sera. This effect was observed within a year after therapy. The reduction in circumoval precipitin content of serum may be used as a test to determine the therapeutic effect of Fuadin or other anti-schistosome drugs. The circumoval precipitin test, therefore, is both of diagnostic and prognostic value.

6. Intradermal response to egg antigen differs from that elicited against adult and cercarial antigens in that a negative reaction is observed in active cases. The skin response becomes positive after treatment of infection.

7. Knowledge of stage-specific antibodies, and the time during the course of infection when their concentrations are highest, is of great importance in the serological diagnosis of schistosomiasis. Stage-specific antibodies are also of great interest in the study of the mechanism of acquired immunity to schistosomiasis.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. D. B. McMullen, Dept. of Medical Zoology, Army Medical Service Graduate School, Washington, D. C. for his suggestions and improvements on the manuscript. Also to Drs. Rurico Diaz Rivera and Francisco Ramos Morales, from the San Juan City Hospital, Santurce, P. R., who made patients available; to Dr. A. M. Stirewalt, Naval Medical Research Institute, Bethesda, Md., for supplying some of the sera from single-sex infections, and to Mrs. Awilda G. Salas and Mr. Luis M. Berrios for their technical assistance.

REFERENCES

Bauer, R., Bauman, P. M., and Salas, Awilda G., paper to be published.

Fairley, N. H., 1951. Schistosomiasis and some of its problems, *Tr. Roy. Soc. Trop. Med. & Hyg.* **45:** 279–303.

Liu Ch'ien, and Bang, F. B., 1950. Agglutination of cercariae of *S. mansoni* by immune sera, *Proc. Soc. Exper. Biol. & Med.* **74:** 68–72.

Oliver-González, J., 1954. Anti-egg precipitin in the serum of humans infected with *S. mansoni*, *J. Infect. Dis.* **95:** July-Aug. pp. 86–91.

Oliver-González, J., and Bauman, P. M., Species specificity of the anti-egg antibody, *J. Infect. Dis., in press.*

Oliver-González, J., Bauman, P. M., and Benenson, A. S., Intradermal response to egg antigen in humans with active and treated (Fuadin) *Schistosoma mansoni* infections, *in press.*

Papirmeister, B., and Bang, F. B., 1948. In vitro action of immune serum on cercariae of *S. mansoni*, *Amer. J. Hyg.* **48:** 74–80.

Vogel, H., and Minning, W., 1948. Hüllenbildung bei Bilharzia—Cercaria im serum bilharzia—infirzierter Tiere and Menschen, *Zentralbl. F. Bakt.*, I Orig., **153:** 99–105.

DISCUSSION OF DR. OLIVER-GONZÁLEZ' PAPER

FREDERIK B. BANG

The Johns Hopkins University School of Hygiene and Public Health

It is a pleasure to discuss Dr. Oliver-González' paper on serological responses to schistosomiasis. Several observers have found, when studying this disease under natural conditions, that there is a decrease in the percentage of individuals who have eggs in their stools as the age of the individuals increases. Presumably, these people are still exposed to infection and many believe that the worms live for an indefinite period of time in the host. Also, the percentage of individuals with enlarged livers decreases with age. This has encouraged the idea that some kind of immunity following repeated infection operates in these hyperendemic areas.

A number of workers, including several within this audience, have developed techniques for studying this problem. They have shown that there is a moderate reduction in the number of worms which developed in animals on reinfection as compared to those receiving the first infection. Perhaps the most outstanding work is that of Vogel and Minning (1945) who studied repeated infections of monkeys for as long a period as ten years. The continued infection did not cause an increase in the number of eggs excreted in the stool but actually caused a reduction or disappearance. These animals were also able to survive doses of cercariae which killed previously uninfected control animals. A few of these immune animals were examined at autopsy and no worms were found.

A number of serological tests have been developed recently in schistosomiasis. I shall not discuss the complement fixation or the precipitin test but will concentrate on the tests which utilize the exposure of some stage of the living worm to serum. If cercariae are placed in fresh immune serum, a precipitate forms around them (Papirmeister *et al.*., 1948). If cercariae are placed in heated immune serum and osmotic changes allowed to occur, a hood will appear around the body and tail of the cercariae (Vogel *et al.*, 1945). If large numbers of cercariae are placed in heated immune sera, they will be agglutinated (Liu *et al.*, 1950). If miracidia are placed in immune sera they are immobilized presumably by the agglutination of the cilia (Senterfit, 1953). All of these reactions occur early in the course of experimental infections and in human infections. We have, as yet, no evidence which allows us to determine whether these are all due to the same antibody and are thus different manifestations of the same reaction or whether different antigens are involved. Differentiation would be possible on the basis of time of development and cross absorption tests. These have not been completed.

Dr. Oliver-González has described a test which involves the exposure of the living eggs to immune serum. The development of a series of finger-like projections from the surface of the eggs is indeed a striking phenomenon. It seems unlikely that the actual processes themselves consist of antigen and antibody but this does not, of course, mean that the reaction has not been started in some

way by such a reaction between antigen and antibody at the egg surface. Dr. Oliver is more aware than the rest of us of the difficulty of measuring the amount of the substance placed in the serum which causes this reaction. He has attempted measurements on the basis of the percentage of eggs which develop this reaction. Presumably there is a variation from one egg batch to another and we are not able to compare this reaction which is directed against the intact egg with the other types of reaction until truly quantitative data are available.

It might be worth-while then to review the points on which I believe most workers are in agreement. First, there is an early increase and a good titer of antibodies to living cercariae and miracidia in the sera of infected animals. Second, there is a lack of specificity in these reactions with the apparent exception of the reaction to the egg which Dr. Oliver has just described. In the hood reaction Kagan (1954) has shown some crossing with *S. douthitti* and we have consistently obtained cross reactions with the three species inhabiting man. Third, there is a cross reaction with the serum from animals and humans who are infected with trichinella. It is possible that some of the antibody causing the latter cross reaction can be absorbed but this has not been demonstrated.

It may also be worth-while to emphasize some of the things we need to know about these reactions. We do not know the relationship to the development of resistance. Vogel and Minning (1945) tested the sera from their immune monkeys against cercariae. They found that the cercariae survived in sera from completely resistant monkeys just as long as they survived in normal sera. They felt that this indicated that the reaction itself is not of importance in immunity. The presence in the sera of antibodies which have a direct effect upon the invading microorganism early in the course of the infection at a time when the organism continues to exist or even multiply is, however, not a phenomenon unique to helminthic infections. In the case of brucellosis, a bacterial disease, there is actually a high positive correlation between the presence of agglutinating antibodies and the presence of bacteria in the blood culture. Typhoid bacilli may be frequently obtained from the blood at a time when agglutinins are present in large amounts. We simply do not know how most of the antibodies operate which are present during the course of many infectious diseases. It is work such as Dr. Oliver has described which will allow us eventually to understand the actual relation between antibodies and resistance.

REFERENCES

1. Papirmeister, B., and Bang, F. B., 1948. The *in vitro* action of immune sera of cercariae of *Schistosoma mansoni*, *Am. J. Hyg.* **48**: 74–80.
2. Kagan, Irving G., 1954. The *in vitro* activity of schistosome ceracriae and miracidia in serum from experimental, natural, and immunized hosts, *Jour. Parasitol.*, **40**: 36.
3. Liu, C., and Bang, F. B., 1950. Agglutination of cercariae of *Schistosoma mansoni* by immune sera, *Proc. Soc. Exp. Biol. & Med.* **74**: 68.
4. Senterfit, L. B., 1953. Immobilization of *Schistosoma mansoni* miracidia by immune serum, *Proc. Soc. Exp. Biol. & Med.* **84**: 5–7.
5. Vogel, and Minning, 1949. Hullenbildung bei Bilharzia Cercarien in Serum bilharzia-infizierter Tiere und Menschen, *Zbl. Bakt.* 1, Orig. **153**: 91–105.

TREATMENT OF SCHISTOSOMIASIS

HARRY MOST

Department of Preventive Medicine, New York University-Bellevue Medical Center, New York, N. Y.

For the past 35 years the principal and most widely used drugs in the treatment of schistosomiasis have been two antimony compounds: tartar emetic introduced by Christopherson (1918) and stibophen (Fuadin) a sulfonated catechol-antimony complex introduced by Khalil *et al.* (1929). Literally tons of these drugs have been used, largely empirically, in the treatment of this infection. The literature dealing with the subject is indeed voluminous, and as one reviews it, one cannot escape the recurring theme of pessimism in relation to the alleged shortcomings of these drugs, and one notes the oft repeated expression of hope for better drugs. The chemists and other laboratory investigators are repeatedly urged to provide such drugs, yet there are few examples of critical stock-taking on the part of the clinicians relative to the actual efficiency of the antimony compounds already available as therapeutic agents. Fortunately opportunities have been provided during the past 10 years to permit the evaluation of these and other compounds in nonendemic areas and under circumstances allowing for long-term post-treatment study, at least in infections due to *S. japonicum* and *S. mansoni*.

It is obviously beyond the scope of this brief presentation to review the vast literature dealing with the treatment of schistosomiasis and to discuss at length the more significant contributions which have been made. Instead, in reading the literature, I have sought for threads which one might weave into a hopeful pattern. Admittedly, this becomes a personal opinion and the result may appear arbitrary. However, the brief comments which follow should provide clinicians at least a basis for reflection in their selection of drugs and treatment schedules in the therapy of schistosomiasis.

The major contributions deal with the relative value of tartar emetic and stibophen against *S. japonicum*, *S. mansoni*, and *S. hematobium*, the optimum schedule of administration and total dosage of these compounds, the parenteral and oral trial of other trivalent as well as pentavalent antimony compounds, and the discovery in Germany during World War II of xanthane and thioxanthane derivatives highly active in experimental primate schistosomiasis.

At the present time tartar emetic in my opinion is the most effective drug in the treatment of the three human schistosomal infections. Total doses of 2.5 gm., 1.8 gm., and 1.5 to 1.75 gm., will cure more than 90 per cent of infections due to *S. japonicum*, *S. mansoni*, and *S. hematobium*, respectively. The principal disadvantages attending the use of tartar emetic are that the drug requires care and experience in administration, is moderately toxic, and must be administered intravenously during a relatively long course requiring numerous injections. Despite these objections, tartar emetic is the drug of choice in the treatment of

infections caused by *S. japonicum*. The pharmacology, toxicity, schedules of administration, and comparative efficiency of tartar emetic and stibophen in this infection have been reported by Most *et al.* (1950), in studies conducted during World War II. They found that a standard course of 40 cc. of stibophen (6.3 per cent solution) cured only about 10 to 15 per cent of patients with *S. japonicum* infections. If the dose was increased to 70 cc., cure rates were only about 25 to 30 per cent, whereas an equivalent amount of antimony contained in 1.45 gm. tartar emetic cured approximately 50 per cent. If the total dose of stibophen on a schedule of 5 cc. every other day is increased to 100 cc. the cures reach only 40 per cent, whereas if the same amount of antimony is given as tartar emetic at least 90 per cent of cures are observed. It is obvious as a result of these observations that tartar emetic is superior to stibophen in the treatment of schistosomiasis caused by *S. japonicum*. It is also apparent that *S. japonicum* is less sensitive to antimony than are either *S. mansoni* or *S. hematobium* because doses of 70 to 100 cc. of stibophen, which cure only 30 to 40 per cent of *S. japonicum*, result in cures of at least 75 per cent of the other infections. Further, if 5 cc. of stibophen are given every day to a total of 100 cc., almost 100 per cent of *S. mansoni* infections may be cured. In support of the latter are two series of Puerto Rican patients treated in a military hospital in this country and in a clinic in New York, and numbering 45 and 176 respectively. In the former there were no failures and in the latter, followed for a year or more during which numerous stools and rectal biopsy specimens were examined, there were 4 per cent failures. It must be emphasized however that a 5 cc. daily schedule of Fuadin injections may be hazardous especially in malnourished underweight individuals. Recently several eaths attributed to the amount of Fuadin given have been observed.

We have also had an opportunity of reevaluating the therapeutic effects of the tartar emetic schedules utilized in the World War II schistosomiasis japonica trials referred to above (Most *et al.*, 1950). One hundred and five patients who received treatment were reexamined in New York 3 to 5 years after completion of therapy. Three to six stool specimens were examined by a variety of concentration techniques in our laboratory as well as in the laboratory of the Army Medical Center. One or more rectal biopsies were obtained and examined fresh and stained after sectioning. A total of 8 failures were found, but of more than 50 patients who had received 1.8 gm. or more of tartar emetic only 2 positives were found. No positives occurred above 2.2 gms.

With regard to so-called intensive treatment, i.e., schedules in which total doses are markedly increased or the period of therapy contracted to a few days, there is evidence that the cure rates in *S. mansoni* and *S. hematobium* infections are significantly enhanced. A two-day course of 6 to 8 injections of sodium antimony tartrate (12 mg./kg. total dose) was introduced by Alves (1945) in Southern Rhodesia. Since then several hundred patients have been treated and followed for several months, cure rates of 75 per cent or more being observed. In our experience this schedule, as well as a continuation of this daily dosage for 5 days, was totally ineffective against *S. japonicum*. Further, trials in Egypt were found so toxic that this method had to be discontinued. Similarly, although 8 cc.

of stibophen daily for 13 days is superior to the standard course of 100 cc. extending over 3 to 4 weeks, the method was too toxic for general use. In short, the so-called intensive schedules, while improving the therapeutic results have been found too toxic and impractical for mass control measures. Perhaps the recent encouraging report of Friedheim *et al.* (1954) in Brazil following the short term use of a new compound, TWSb (antimony-a,a′-dimercapto-potassium succinate) may, if the initial experience of low toxicity and a high order of definitive cures is confirmed following extensive field trials, resolve the shortcomings of the so-called intensive schedules insofar as schistosomiasis mansoni and probably schistosomiasis hematobium are concerned. Nevertheless, the data suggest the desirability of further clinical trials with this compound.

Other trivalent and pentavalent antimony compounds (ethylstibamine, stibamine glucoside, urea stibamine, anthiomaline and stibanose) have been found moderately effective in *S. mansoni* infections and ineffective in *S. japonicum*. On the whole, on the basis of toxicity in some instances and of the need for intravenous medications in others, it is questionable whether any offer advantages over stibophen. Antimony compounds, notably antimony trigulconate and butyl antimony gallate, while moderately effective in animal trials orally, have been almost totally ineffective and too toxic for human use by mouth.

During World War II, Kikuth, Gönnert & Mauss (1946) developed a series of xanthone and thioxanthone derivatives which were effective orally in experimental schistosomiasis. These compounds, principally miracil, miracil D, their naphthoate, salicylate, and other derivatives, have had limited or extensive trials in various parts of the world. A most interesting report presenting original work as well as a review of the status of these agents has been made recently by Newsome (1951). Apparently most investigators who have studied these drugs have agreed as to their clinical effectiveness, principally in *S. haematobium*, and less so in *S. mansoni* infections. However, there have been major differences in therapeutic results and toxic experiences, and in the light of recent studies, these appear to be related to the preparations or derivatives of miracil studied, the total dose and schedules of administration, differences in severity of infection and exposure to re-infection in various geographic areas, and, most important, differences in criteria of cure. Newsome, on the basis of his observations, which included hatching and rectal biopsy techniques in evaluating cure after treatment, concluded that "miracil D has a marked curative effect on *S. haematobium* while the effect on *S. mansoni* is perhaps less pronounced". Calculated actual cure rates appear to be 39 per cent and 27 per cent respectively for these infections, although clinically most patients appear to be cured. The results in Africa and South America appear to be better than in Egypt, but in this country the results would probably be better if the drug could be given as two three-day courses in the order of 100 to 125 mg./kg. Spingarn (1954) in New York has treated 9 patients with larger doses (4 to 14.4 gm. total administered in two three day courses separated by a rest period of 3 days). Only 7 completed this schedule and 4 who were followed for more than 6 months were apparently cured. However, all patients experienced severe toxic reactions notably related to the central

nervous system and manifested chiefly by confusion, hallucination and psychosis. These observations indicate the potential curative properties of this class of compounds which in their present form are too toxic for optimum use. Coated tablets have been found less toxic than the uncoated hydrochloride of miracil, and undesirable side effects are also reduced if the naphthoate or salicylate derivates are used. These compounds require additional clinical trials.

From the foregoing, it appears that tartar emetic administered intravenously in total doses of 2.5 gm. according to the schedule of Most *et al.* (1950) is the drug of choice in the treatment of *S. japonicum* infections. This schedule should cure at least 95 per cent of patients. If, because of toxic manifestations or the necessarily prolonged period of hospitalization, this schedule is not feasible, then daily intramuscular injections of 5 to 8 cc. of stibophen for at least 20 consecutive days are recommended. The latter course of treatment will cure approximately 60 to 75 per cent of the infections, depending upon the size of the daily dose tolerated and the total dose given. In the treatment of individual cases of *S. haematobium* and *S. mansoni* infections, 5 cc. of stibophen intramuscularly daily are recommended with total doses of 75 cc. and 100 cc. respectively for the above infections from the standpoint of definitive cures approaching 100 per cent. For alleviation of symptoms or mass control schedules for large population groups, the miracil derivatives are recommended with the reservation that the actual cures will be low. The so-called intensive two- to three-day antimony schedules and the oral antimonials presently available are either too toxic or insufficiently active to favor their adoption except for experimental purposes.

In closing one can draw an analogy between the current status of schistosomiasis treatment and the early arsenotherapy of syphilis. In spite of the potential and actual toxicity of salvarsan and, later, neosalvarsan and mapharsen, the prolonged and repeated intravenous and intramuscular courses of treatment lasting a year or more at first and later shortened by so called intensive continuous treatment, and the numerous serious reactions, these drugs were hailed as a boon in the control of a disease which had plagued mankind for centuries. Perhaps the ideal nontoxic oral preparation which is curative for all forms of human schistosomiasis will eventually be found and be applicable for individual and mass treatment; or perhaps the biological or chemical control of the snail, together with educational and other public health measures, will eradicate the infection. But until we find for schistosomiasis the equivalent of the present penicillin treatment for syphilis we should capitalize on the well documented experiences we already have and approach each case with the knowledge that definitive cure can be accomplished.

REFERENCES

ALVES, W. M., 1945. Intensive treatment of schistosomiasis with antimony (preliminary note), *S. African Med. J.* **19**: 171.

CHRISTOPHERSON, J. B., 1918. The successful use of antimony in bilharziasis, *Lancet* **2**: 325–327.

FRIEDHEIM, E. A. H., DA SILVA, J. R., AND MARTINS, A., 1954. Treatment of schistosomiasis

mansoni with antimony-a,a'-dimercapto-potassium succinate (TWSb), *Am. J. Trop. Med.* **3:** 715.

KHALIL, M., *et al.*, 1929. Die Behandlung der Schistosomiasis mit intramuskularen "Fuadin" Injektionen, *Deut. med. Wochsch.* **55:** 1125.

KIKUTH, W., GÖNNERT, R., AND MAUSS, H., 1946. Miracil ein neues chemotherapeuticum gegen die Darmbilharziose, Naturwissenschaften. **33:** 253.

MOST, H., KANE, C. A., LAVIETES, P. H., SCHROEDER, E. F., BEHM, A., BLUM, L., KATZIN, B., AND HAYMAN, J. H., JR., 1950. Schistosomiasis japonica in American military personnel: clinical studies of 600 cases during the first year after infection, *Am. J. Trop. Med.* **30:** 239–299.

NEWSOME, J., 1951. Recent investigations into treatment of schistosomiasis by miracil D in Egypt, Trans. Roy. Soc. Trop. Med. Hyg. **44:** 611.

SPINGARN, C., 1954. Personal communication from the Mount Sinai Hospital, New York, N. Y.

DISCUSSION OF DR. HARRY MOST'S REPORT ON THERAPY OF SCHISTOSOMIASIS

HAMILTON H. ANDERSON, M.D.[1]

The use of tartar emetic (antimony and potassium tartrate) in preference to Fuadin has been emphasized properly, in combatting schistosomal infections. Of the clinical forms mentioned, *S. japonicum* infections are most resistant to therapy, and also are not infrequently overlooked in differential diagnosis. Four deaths have occurred fairly recently in San Francisco in personnel returned from overseas (personal communication from Dr. H. G. Johnstone).

Not all physicians using tartar emetic are aware, I believe, of the need for beginning with very small doses and after some tolerance to antimony has developed, build up the dose to much higher levels than originally possible. This technic is based on sound pharmacologic evidence in animals.

Dr. C. Pak (now Surgeon General of the Korean Navy), when working in Shanghai (before the Japanese-American conflict) at the Henry Lester Institute for Medical Research, found that rats or mice, given a dose equivalent to ⅓ of the lethal amount of an antimonial, within 5 to 48 hours can be given a surely fatal dose of the same antimonial without harm.

With respect to Miracil-D, I have had the opportunity to review with Dr. Watson his experience with this agent against schistosomiasis in the Middle East. It is my understanding that while the drug has some effect on *S. haematobium* it is not too active against other schistosomal infections. Further, Dr. Watson, one of the first to engage in field trials with this agent, did not believe that Miracil-D should be employed routinely by the average physician, because of its undue toxicity even with recommended therapeutic amounts.

Finally, it is my opinion that the criteria for cure of schistosomal infections in man should be more clearly defined and generally understood. Disappearance of ova from the feces in *S. japonicum* and *S. mansoni* infections particularly, and rectal biopsy, are not adequate criteria alone. One cannot be certain, on the basis of such criteria, that schistosomal infection elsewhere, notably in the liver and brain, has been cleared. Liver biopsy might be employed more frequently to assess the status of the liver in individuals about to be discharged as cleared of their infection.

[1] University of California, San Francisco.

SOME CONTRIBUTIONS OF TROPICAL PEOPLES IN THE PRESERVATION OF FOOD MATERIALS

WILLIAM H. ADOLPH

Professor of Nutrition, American University of Beirut[1]

The Occident is making rapid advances in the field of food preservation. We have been inclined however to overlook the contribution which peoples of the past in other areas have made in this field. These early discoveries, largely unconscious and possibly the result of blind experiment, should not be overlooked.

Early agricultural man was limited in his choice of food materials, but he made a number of food choices which we now realize were rather intelligent ones. While there is little evidence that instinct plays a significant role in human nutrition, it does seem that the dietary developed by agricultural man was just about as good as could be expected under the circumstances. The early accomplishments in food preservation quite probably are part of his striving toward an effective diet.

Man found it necessary for example to store food materials till the next harvest furnished a new supply. He constructed therefore simple underground storage cellars, where, as in North China, vegetable materials such as sweet potatoes, cabbage and also fruit are kept through the winter and at the same time subjected to a maturing process. The treatment of persimmons in North China is a good example of simple food processing. Almost all the early peoples discovered that certain fruits and vegetables could be dried against future use. Examples of simple desiccation or dehydration are raisins, dried apricots, dried sweet potato chips, dried tomato juice, etc.

But in tropical and semi-tropical areas the problem of storing food supplies is more difficult. Nevertheless the people of these areas did attack this problem, and their early accomplishments are worthy of study. Such study must be made before the early methods disappear or before they are replaced by the mechanical devices of modern food industry. A few of the more intriguing examples are presented below.

EARLY FOOD PROCESSING

Parboiled wheat (*burghul*) is perhaps the most striking of the processed food materials developed in the Near East; its preparation has been described elsewhere (Shammas *et al.*, 1954). In warm semitropical areas, whole wheat flour and even wheat itself are subject to spoilage. But by the parboiling process, which involves a brief cooking followed by rapid drying in the hot sun, a product is formed which can be kept indefinitely. Moreover in the process a large part of the vitamins are retained; it is analogous to the better known parboiled rice.

[1] Present address: Yale University Nutrition Laboratory, New Haven, Conn.

The early inhabitants of the Near East who discovered *burghul* are to be commended. White flour, in contrast, is the product which the Occident has developed to facilitate storage. But in the process of manufacturing white flour, thiamine has been removed; its absence is related to the occurrence of beriberi. We have seen no reports of a thiamine deficiency disease in the Near East.

Dried Milk (*Jubjub*). The dry, hot climate of the Near East also made possible the development of dried milk, a material which apparently dates back to early history. Of the several forms of dried milk known, *jubjub* is one of the more common; it is prepared by treating curdled milk with salt and then drying in the hot sun. The resulting dry product comes on the market in white lumps, containing approximately 10 per cent of salt.

Butter Oil (*Ghee, Samneh*). Without refrigeration, butter in tropical countries rapidly becomes rancid. It is not surprising to find therefore that butter is largely replaced by butter oil (*samneh* in the Near East; *ghee* in India), with keeping qualities far superior to butter. Butter oil, frequently referred to as clarified butter, is prepared in the Near East using curdled milk followed by shaking and churning in goat-skin bags. The cream layer is then separated, and boiled for about half an hour with parboiled wheat. A clear layer of butter fat results which is decanted and may be treated with parboiled wheat a second time. The final product is stored in tightly covered jars; coloring matter (safron) and spices may be added. In the clarifying process both moisture and protein are removed and the product when carefully prepared may be kept for a year or more. It does not require refrigeration.

PRODUCTS OF MICROBIAL ACTION

Methods of freezing and refrigeration have been widely used in the temperate zone for the storage of food materials, but the wide use of these methods has not been possible in tropical areas where the majority of the population cannot afford modern refrigerator equipment and ice is not generally obtainable. In regions where the climate is both hot and wet, the prevention of food spoilage is a serious problem. As a result, tropical areas in some cases have turned to microorganisms as their preserving agent. One of the best examples of this is in the use of *leban* (*yoghurt*). Other examples are *nuoc mam* and *bagoong*.

Leban, or *yoghurt*, has been widely advertised. It is the form of curdled milk produced by the addition of an *acidophilus* culture to milk; such cultures and their products have been studied by Burkey (1948). The peoples of the Near East use *leban* extensively and believe that its use in the diet insures a healthy digestive tract. It may be prepared not only from fresh milk but also from dried milk powder. But the other aspect of the problem, namely that the conversion to *leban* (*yoghurt*) is essentially a process for preserving milk, is largely overlooked. *Acidophilus* prevents the growth of other organisms that would cause putrefaction. Milk could not be so widely and effectively used as food in the Near East were the process of conversion into *leban* not possible.

The eastern Mediterranean area is noted not only for the wide use of *leban*

(*yoghurt*), but also for the use of a number of *leban* products. Freshly made *leban* may be mixed with parboiled wheat; the resultant mixture when dried and powdered resembles dried milk in both odor and texture. This is known as *kishk*, and is eaten as a soup or gruel. Both *kishk* and *jubjub*, mentioned above, may be regarded as the forerunners of modern dried milk.

Fish Sauce (*Nuoc Mam*). Fresh fish constitute an important food material in southeastern Asia. There are however many regions far removed from the source of supply, so that the prevention of spoilage during transport becomes necessary. This has been effectively accomplished with the help of microorganisms. *Nuoc mam* is the best known of the fish sauces developed in this way.

For the making of *nuoc mam*, the fish are pressed and salted, usually in earthen pots, which are then sealed and buried. After several months the containers are opened and the clear, brown liquid decanted. This liquid is *nuoc mam*. It is of course rich in salt but it contains also a high percentage of amino acids resulting from the hydrolysis of the protein. The nitrogen content (soluble nitrogen) is about 10–15 grams per liter. The original fish material is rich in the enzymes contained in the fish entrails, so that the product must be in part the result of enzymatic action. Food products of this sort may be compared to modern protein hydrolyzates, and may be expected to have a corresponding nutritive value.

Fish Paste. Bagoong is typical of a large variety of fish pastes made in Indo-China and Indonesia. The fish is salted in vats, usually employing less salt than in the manufacture of *nuoc mam*, and the mixture is then allowed to stand for several months to ripen. The resulting paste may contain 10–15 percent of protein. The character of the product varies depending upon the technique used in its manufacture. *Bagoong* is probably largely the result of bacterial action and may be regarded as a rather effective device for preserving fish. Both *nuoc mam* and *bagoong* are important sources of food nitrogen in the dietary of this area (Van Veen, 1953).

MORE DATA NEEDED

We have not discussed the more commonly known products of both the temperate and tropical regions: the cheeses, sauerkraut, etc., nor the soy sauce of the Far East. The foods mentioned above are presented merely as examples of products found in tropical countries which seem to us to represent distinct contributions in the realm of food preservation. They may be the result of blind experiment, but this in no sense detracts from their importance as discoveries. These foods are too often classed as eclectic and as dietetic curiosities. They cannot be altogether the product of chance, but must be the result of efforts to fill real nutritional needs. These, as well as similar types of products from other parts of the tropical world, need further study. These prehistoric examples of food processing not only call attention to the needs which mankind early attempted to meet, but they frequently suggest the manner in which such problems even today can be solved.

CONCLUSION

A few of the more important contributions of the people of the high temperature areas in the field of food processing are described. These early discoveries are worthy of more detailed study.

REFERENCES

BURKEY, L. A., 1948. Bulgaricus and acidophilus cultured milks, *U.S.D.A. Bur. Dairy Ind.* BDIM-Inf-16, pp. 10.

SHAMMAS, E. I., AND ADOLPH, W. H., 1954. Parboiled wheat in the Near East. *J. Am. Dietet. Assoc.* **30**: 982–983.

VAN VEEN, A. G., 1953. Fish Preservation in Southeast Asia. *Adv. Food Res.* **4**: 209–231.

EPIDEMIOLOGY OF AMEBIASIS: POSSIBLE INFLUENCE OF WATER SUPPLY, COINCIDENT WITH DIVERSE FEATURES OF TERRAIN, ON THE OCCURRENCE OF INTESTINAL PROTOZOA

DALE E. WYKOFF,[1] J. R. C. FONSECA AND L. S. RITCHIE

Department of Medical Zoology,[2] 406th Medical General Laboratory

Although water has been implicated in epidemic amebiasis as many times as other modes of transmission collectively, its relative importance to endemic transmission of intestinal protozoa is essentially unknown. Hunter *et al.* (1950, 1951) reported circumstances in Fukui and Yamanashi Prefectures, Japan, where different sources of drinking water seemingly accounted for differences in the incidence of intestinal protozoan infection. Communities on level terrain with open wells as a source of water had fewer infections than mountain villages where, because terrain precluded digging of wells, water was obtained from streams.

These observations have been further evaluated by an extensive survey of villages in the broad Kofu valley and surrounding mountains, Yamanashi Prefecture, Japan. In addition, a comparison of findings for villages at different levels in several narrow valleys has been made on the assumption that the degree of fecal pollution would be greater downriver.

The protozoan infections in a particular population and the differences of incidence for several populations are probably due to the accumulative effect and possible interaction of several modes of transmission. The relative importance of the several factors, which undoubtedly varies in different areas, has never been successfully measured, and in this investigation is only postulated on circumstantial evidence. For villages currently investigated, the source of drinking water was the most evident variable and probably constituted the major factor for difference of protozoan infection rates in populations with otherwise very similar living conditions.

PROCEDURES

The terrain features of the Kofu Valley, Yamanashi Prefecture, were ideal for this investigation. The main or primary valley, with a floor area of about 100 square miles, is virtually surrounded by mountains of about 5,000 feet above sea level, into which radiate numerous narrow secondary valleys. A group of villages in several of the latter were examined and the findings compared with those of population centers located on the floor of the main valley. In the valley, open wells served as the principal source of drinking water, while hill communities were necessarily dependent upon streams and rivers. The areas of interest were within a radius of eight miles and involved only rural populations with very similar modes and habits of living and comparable standards of edu-

[1] Present address: AMSGS, Walter Reed Army Medical Center, Washington 12, D. C.
[2] APO 500, c/o Postmaster San Francisco, California.

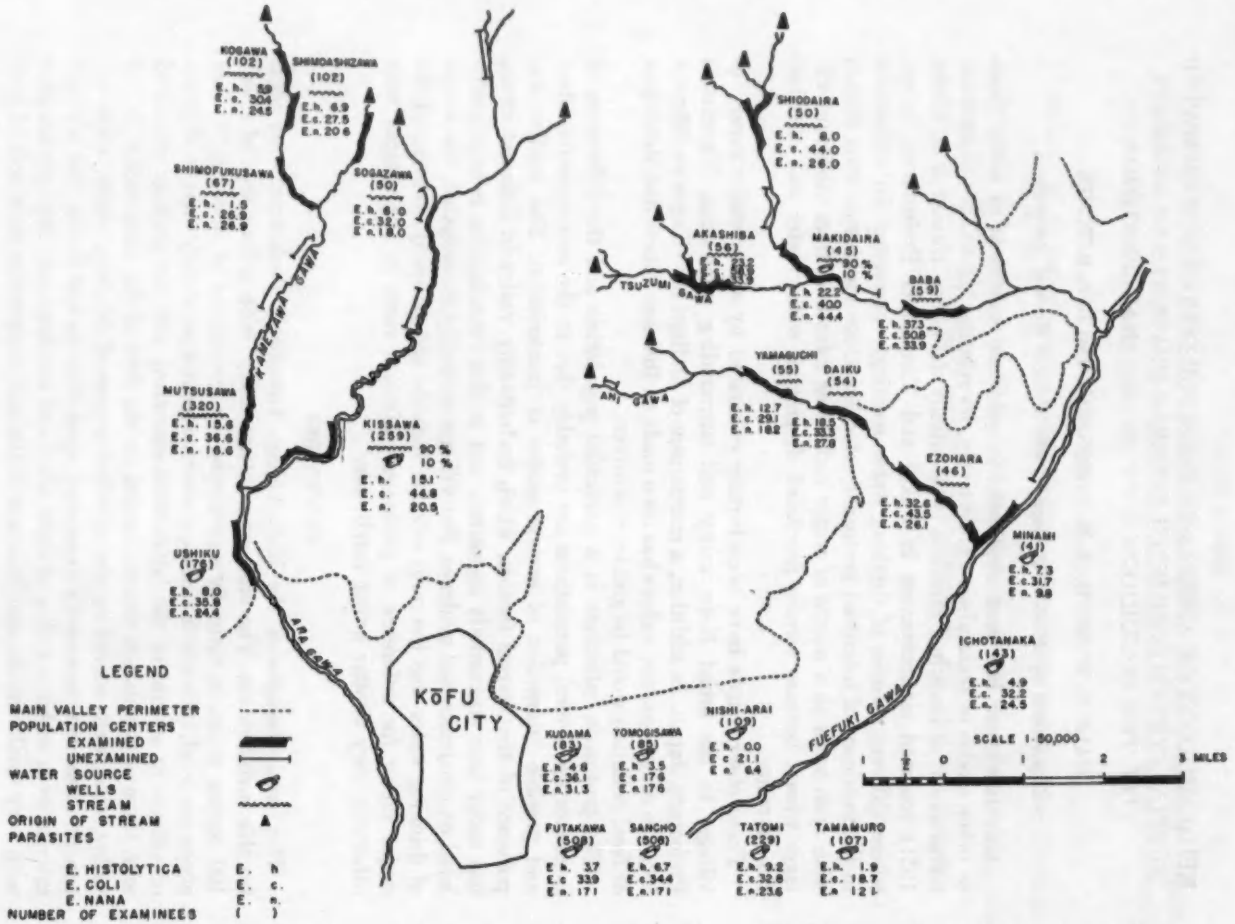


FIG. 1. Map of Villages in Yamanashi Prefecture, Japan, surveyed for epidemiologic studies of intestinal protozoa.

cation. The water supply and its potential contamination constituted the fundamental difference.

Of the three secondary valleys selected, one was of the Ara River watershed, northwest of Kofu City, and the other two were of the Fuefuki River watershed northeast of this city. A map (Figure 1) shows the relative location of hill villages examined, intervening populations not examined, and the river systems. The geographical distribution of the villages on the floor of the main valley is not shown, but they are arranged conveniently at the bottom of the map with associated incidence percentages. The small numbers of persons examined in some hill villages constituted 80 per cent of the population. Entire family groups were utilized in the survey which, it was assumed, would afford uniform numbers of the sexes and the various age groups.

Single-stool examinations, using the formalin-ether procedure (Ritchie, 1948) constituted the basis for the present study.

FINDINGS AND DISCUSSION

Incidence data are organized in Table 1 and Figure 1. The occurrence of *Endamoeba histolytica* was significantly greater ($P > .0001$) in the hill communities where the overall incidence among 1265 persons was 14.8 per cent, in contrast to 5.4 per cent for 1989 persons living on flat areas of the primary valley. Similarly, statistically significant differences occurred for *E. coli* ($P = .005$) and *Endolimax nana* ($P = .0006$); however, these differences did not approach a 2:1 ratio which was exceeded by *E. histolytica*.

In the Fuefuki watershed, northeast of Kofu City, populations of two tributaries were examined. In general, the incidence of *E. histolytica* increased steadily downstream from the headwater villages, which is consistent with an expected increase of fecal contamination. There was no apparent explanation for the exception at Akashiba, but this appears more readily admissible on the basis of the water-borne theory of transmission. The incidence of *E. histolytica*, after rising steadily in successive villages to a maximum of 37.3 per cent at Baba and 32.6 per cent at Ezohara, dropped suddenly to 7.3 per cent at Minami, located below the junction of the two tributaries with the Fuefuki River proper, where wells were in use. At the four headwater population centers of the Ara River (Kogawa, Shimoashizawa, Shimofukusawa and Sogozawa), northwest of Kofu City, the incidence of *E. histolytica* was about 5 per cent. Down river at Mutsusawa the figure was 15.6 per cent, but below Mutsusawa at Ushiku on the valley floor where wells were used, the incidence was only 8 per cent (Fig. 1). Corresponding observations on *E. coli* and *E. nana* indicated that their occurrence in the two watersheds fluctuated independently of location. The incidence of all three protozoa was distinctly higher in the Fuefuki than in the Ara watershed (Table 1).

The dependence of people in the secondary valleys on streams and irrigation ditches for drinking water afforded a reasonable explanation for the higher incidence of protozoa which prevailed. Excessive pollution was inevitable due to runoff of rainfall and irrigation waters, use of streams for laundering, and rins-

TABLE 1

Protozoan infections in populations with different domestic water supplies

	Number persons examined	Number and per cent infected					
		E. histolytica		E. coli		E. nana	
		No.	%	No.	%	No.	%
Hill villages, Ara watershed							
Kogawa	102	6	5.9	31	30.4	25	24.5
Shimoashizawa	102	7	6.9	28	27.5	21	20.6
Shimofukusawa	67	1	1.5	18	26.9	18	26.9
Sogazawa	50	3	6.0	16	32.0	9	18.0
Mutsusawa	320	50	15.6	117	36.6	53	16.6
Kissawa	259	39	15.1	116	44.8	53	20.5
Sub totals	900	106	11.8	326	36.2	179	19.9
Hill villages, Fuefuki watershed							
Shiodaira	50	4	8.0	22	44.0	13	26.0
Akashiba	56	13	23.2	33	58.9	19	33.9
Makidaira	45	10	22.2	18	40.0	20	44.4
Baba	59	22	37.3	30	50.8	20	33.9
Yamaguchi	55	7	12.7	16	29.1	10	18.2
Daiku	54	10	18.5	18	33.3	15	27.8
Ezohara	46	15	32.6	20	43.5	12	26.1
Sub totals	365	81	22.2	157	43.0	109	29.9
Totals	1265	187	14.8	483	38.2	288	22.8
Villages on floor of main valley							
Ushiku	176	14	8.0	63	35.8	43	24.4
Kudama	83	4	4.8	30	36.1	26	31.3
Yomogisawa	85	3	3.5	15	17.6	15	17.6
Nishiarai	109	0	0.0	23	21.1	7	6.4
Tamamura	107	2	1.9	20	18.7	13	12.1
Futakawa*	508	19	3.7	172	33.9	87	17.1
Sancho*	508	34	6.7	175	34.4	87	17.1
Tatomi	229	21	9.2	75	32.8	54	23.6
Ichotanaka	143	7	4.9	46	32.2	35	24.5
Minami	41	3	7.3	13	31.7	4	9.8
Totals	1989	107	5.4	632	31.8	371	18.7

* Taken from Hunter, Ritchie, Kaufman, Pan, Yokogawa, Ishii and Szewczak (1951).

ing of night soil containers. Some protection against the threat of exposure undoubtedly results from the tea-drinking tradition of the Japanese.

The percentage figures of 14.8, 38.2 and 22.8 for *E. histolytica*, *E. coli* and *E. nana* respectively in the hill areas constituted an occurrence ratio of approximately 1:3:2, while for communities of the primary valley the ratio was about

1:6:4. It appears, then, that conditions of transmission in the area of flat terrain were more favorable for *E. coli* and *E. nana*, while those associated with the hills enhanced transmission of *E. histolytica* without affecting the other two. As an explanation, the idea is proposed that the several modes of transmission are not equally effective for these three protozoa. In an area where the role of water is of reduced importance, such as indicated here for flat terrain, the combination of direct and food handler transmission might then play a primary role. Coincident with the latter the influence of cyst drying would be introduced, which according to Reardon *et al.* (1952) is more damaging to *E. histolytica* than *E. coli*. On the other hand, where water is of greater importance, as is indicated for the hilly areas, transmission of *E. histolytica* might be enhanced and the incidence increased out of proportion to the other amebas. Also consistent with this assumption is the observation that the incidence of *E. histolytica* tended to be higher downriver where pollution might be expected to be greater, while *E. coli* and *E. nana* fluctuated irrespective of location.

The possible influence of factors other than water should be considered. Although in general the living conditions were similar, the economic status of the hill villages may have been a little lower. This is a difference which might foster a lower level of household sanitation and personal hygiene, with a related increase in direct and food-handler transmission. Initially, an attempt was made to evaluate the cleanliness of the households included in this study, but satisfactory criteria of distinction were not recognized; indeed there is some doubt whether standards of living encompass factors which are critical in the transmission of protozoa. Milam and Meleney (1931) did not detect any direct relationship between the occurrence of *E. histolytica* and apparent family cleanliness. In a comparison of two communities in Tennessee, Jones *et al.* (1954) noted that conditions of sanitation and cleanliness were superior in the community which had the higher incidence of protozoan infections. Although these same authors (Eyles *et al.*, 1953) reported that protozoa were less apt to be found in "clean" families than in those designated "fair" and "dirty", they found that the incidence of the white population with a much higher standard of living equalled that of the negro population. It seems very doubtful, then, that sanitation and personal hygiene represented the primary factor which accounted for the differences of incidence noted in this investigation.

Flies are highly prevalent throughout Japan with toilet receptacles, night soil storage tanks and manure piles ever present as breeding sites. It was initially assumed that flies could be considered a common denominator in relation to the occurrence of protozoa in the areas under consideration, but a preliminary survey of fly population by the grill-count method of Schudder (1947) revealed a greater density in the hill communities. Repeated sampling extending over several years is considered necessary before this observation can be considered valid. Although the observations of Frye and Meleney (1932), Harris and Downs (1946) and Pipkin (1949) show conclusively that flies constitute a likely means of transmitting intestinal protozoa, the relative importance of this mode of transmission has never been conclusively established.

Contrasting incidence of *E. histolytica* for hill and valley areas was not significantly influenced by variations in sex and age distribution. The data showed that this bias had been adequately controlled by utilizing entire family groups in the survey.

Factors other than water may have contributed to the higher incidence of protozoa in the hill areas; however, existing circumstances and the apparent importance of water in epidemic amebiasis seem to warrant the assumption that water was the major factor of difference in producing a high endemic level of *E. histolytica*. If it is generally true that communities of mountainous areas in Japan are highly endemic for this protozoan, a sizeable portion of its population is subjected to the disease-potential of amebiasis.

SUMMARY

The observations presented indicate that the incidence of protozoan infections was lower on the floor of the Kofu Valley, Yamanashi Prefecture, Japan, where drinking water was obtainable from open wells, than in secondary valleys of surrounding mountains where the primary source of water was either a river or an irrigation stream. For *Endamoeba histolytica* the incidence tended to increase from upper to lower levels of the valley, in agreement with an expected increase in fecal pollution. Fluctuations in the incidence of *E. coli* and *E. nana* which were irrespective of valley levels, and a disproportionate increase in the rate of *E. histolytica* in the hill communities prompt the speculation that the several modes of protozoan transmission may not be equally effective for *E. histolytica* and the other amebas. Since cyst drying has been shown to be less damaging to *E. coli* than *E. histolytica*, the latter may be more dependent on water transmission. Although other factors such as direct and food handler transmission and flies may have contributed to the increased incidence of protozoa in hill villages, it is believed that water was the major factor of difference.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation for the approval and cooperation of Dr. S. Mitsui, Chief of the Public Health Department of Yamanashi Prefecture; Dr. S. Sugiura, Chief of the Department of Schistosomiasis, Yamanashi Medical Research Institute; Dr. Y. Komiya, National Institute of Health, Tokyo; and Dr. M. Yokogawa, Institute of Public Health, Tokyo. Collection of specimens was made possible by the willing assistance of Mr. T. Yamaguchi and Mr. S. Komiyama of the Public Health Department of Yamanashi and officials of the various villages. Expert technical assistance of the following is gratefully acknowledged: Y. Hishinuma, S. Asakura, H. Kutsumi, S. Yamaguchi, Cpl. R. R. Williams and Cpl. T. J. Styles. Finally, the authors are grateful to Major L. P. Frick, Chief, Department of Medical Zoology, 406th Medical General Laboratory, for correcting the manuscript.

REFERENCES

EYLES, D. E., JONES, F. E., AND SMITH, C. S., 1953. A study of *Endamoeba histolytica* and other intestinal parasites in a rural west Tennessee community, *Am. J. Trop. Med. and Hyg.* **2**: 173–190.

FRYE, W. W., AND MELENEY, H. E., 1932. Investigations of *Endamoeba histolytica* and other intestinal protozoa in Tennessee. IV. A study of flies, rats, mice and some domestic animals as possible carriers of the intestinal protozoa of man in a rural community, *Am. J. Hyg.* **16**: 729–749.

HARRIS, A. H., AND DOWN, H. A., 1946. Studies of the dissemination of cysts and ova of human intestinal parasites by flies in various locations on Guam, *Am. J. Trop. Med.* **26**: 789–800.

HUNTER, G. W. III, RITCHIE, L. S., NAGANO, K., AND ISHII, N., 1950. Parasitological studies in the Far East. II. An epidemiologic survey in Fukui Prefecture, Honshu, Japan, *Jap. Med. J.* **3**: 359–364.

HUNTER, G. W., III, RITCHIE, L. S., KAUFMAN, E. H., PAN, C., YOKOGAWA, M., ISHII, N., AND SZEWCZAK, J. T., 1951. Parasitological studies in the Far East. IV. An epidemiologic survey in Yamanashi Prefecture, Honshu, Japan, *Jap. Med. J.* **4**: 113–124.

MILAM, D. F., AND MELENEY, H. E., 1931. Investigations of *Endamoeba histolytica* and other intestinal protozoa in Tennessee. II. An epidemiological study of amebiasis in a rural community, *Am. J. Hyg.* **14**: 325–336.

PIPKIN, A. C., 1949. Experimental studies on the role of filth flies in the transmission of *Endamoeba histolytica*, *Am. J. Hyg.* **49**: 255–275.

REARDON, L. V., VERDER, E., AND REES, C. W., 1952. The cultural requirements of *Endamoeba coli* and the comparative effects of drying on the cysts of *E. coli* and *E. histolytica*, *Am. J. Trop. Med. and Hyg.* **1**: 155–161.

RITCHIE, L. S., 1948. An ether sedimentation technic for routine stool examinations, *Bull. U. S. Army Med. Dept.* **8**: 326.

SCUDDER, H. I., 1947. A new technique for sampling the density of housefly populations, *Pub. Health Rep.* **62**: 681–686.

THE USE OF PENICILLIN AND STREPTOMYCIN IN THE ROUTINE CULTIVATION OF AMEBAE FROM FECAL SPECIMENS

LOIS NORMAN AND M. M. BROOKE

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia

Penicillin and streptomycin because of their low amebicidal potentials (Balamuth and Wieboldt, 1946; Phillips, 1951; Bradin and Hansen, 1950) have been used as bacteriostatic agents in cultures of intestinal protozoa. Balamuth (1948) used each of these antibiotics to control the multiplication of bacteria and thus enhance the growth of several species of amebae in vitro. Spingarn and Edelman (1947, 1948, 1952) in a series of papers, and Michael and Cooray 1952) have shown that growth was improved when either penicillin or streptomycin were added to the medium before inoculation of fecal material containing *Entamoeba histolytica*. Inoki *et al.* (1953) and Faust (1952) recommend the use of both antibiotics in the medium for diagnostic cultures, but reports of comparative results obtained in routine diagnostic work have not been found in the literature.

Since 1948, various combinations and amounts of antibiotics have been used in the parasitology laboratories of the Communicable Disease Center as bacteriostatic agents both in the maintenance of stock cultures of several species of intestinal protozoa and in diagnostic work. A routine procedure for processing fecal specimens has been adopted in which duplicate inoculations are made into medium containing relatively small amounts of both penicillin and streptomycin and into medium without antibotics. Results obtained by cultivation of a group of 120 specimens mailed to the laboratory in 1950 showed 18 cultures positive for *E. histolytica* in the medium with penicillin and streptomycin as compared to 7 positive in cultures without antibiotics. The following report presents the results obtained using this established procedure for the isolation of amebae from fecal specimens collected during two surveys. Inasmuch as the specimens were also examined by other techniques, opportunity was afforded to determine the relative efficiency of this cultivation routine in the diagnosis of intestinal amebae.

MATERIALS AND METHODS

In Survey I, a group of 65 normally passed stool specimens were mailed to the laboratory from a mental institution in a midwestern state. Each specimen has been divided while fresh into three portions: one portion was mixed with formalin to be used for concentration techniques; one was mixed with PVA-fixative for the preparation of stained smears; and one was left without preservative to be used for cultivation. All of these specimens were 2 to 4 days old before inoculation into the culture medium. In Survey II, specimens were collected from 347 patients in a local Veterans Administration Hospital. All specimens were post-cathartic and usually the third specimen collected was used for culti-

vation. They were less than 6 hours old when inoculated into the medium. All of the specimens were also immediately examined microscopically, and a portion of each mixed with PVA-fixative so that smears for permanent staining could be made at a more convenient time. The rest of the specimen was used for formalin-ether sedimentation (Ritchie, 1948).

Modified Boeck and Drbohlav's medium (LER) was prepared from fresh eggs each week and each slant was overlayed with 5 ml. of buffered Locke's solution without serum (Reardon, 1939). Sterile rice powder (Difco) was added immediately before use. A stock mixture of solutions of penicillin sodium and streptomycin sulfate in buffered Locke's was prepared so that 0.1 ml. contained 1,250 units of each antibiotic. The addition of this amount to each tube of medium gave a final concentration of 250 units of each antibiotic per ml. of overlay. The stock solution was stored at −10°C. until needed and refrozen after use.

Samples from each fecal specimen were inoculated into two tubes of medium, one of which contained the antibiotic mixture, and one of which did not (called Stock medium). After 24 hours incubation at 37°C., small amounts of sediment from each tube were examined microscopically. Quensel's (Svensson, 1935) or V-W-O (Velat-Weinstein-Otto, 1950) staining solutions were used to help find and identify the trophozoites. Approximately half of the sediment in each tube was transferred to tubes of medium without antibiotics. Wet mounts of these subcultures were examined after 48 hours incubation, and then all the remaining sediment in each subculture tube was mixed with 2 or 3 times its volume of PVA-fixative (Brooke and Goldman, 1949). Films were made from each, dried thoroughly, and stained by Heidenhein's iron hematoxylin technique. Each stained smear was examined microscopically for a minimum of 10 minutes.

The stool specimens were also subjected to routine examinations by other individuals in a separate laboratory. The examinations on all specimens included: (a) concentration by the formalin-ether sedimentation technique (abbreviated to FE Sed.); and (b) iron-hematoxylin stained smears. Saline and iodine temporary wet mounts on the 347 specimens of Survey II were also made. The examinations were conducted without collaboration between the two laboratories.

RESULTS

Effect of the use of antibiotics on the cultivation of amebae. The effect of the addition of penicillin and streptomycin to the medium on the number of infections demonstrated by cultivation is presented in Table 1. In the specimens from Survey I, 78 infections were found in the cultures treated with antibiotics as compared to 37 in the cultures not treated. Since 10 cultures were positive in the stock medium that were negative in the medium with antibiotics, a total of 88 infections were found by examination of both. The use of penicillin and streptomycin increased the number of amebic infections detected in Survey II, but to a lesser degree—141 with antibiotics and 109 without. In general, growth of each species of ameba occurred more often in the medium containing antibiotics. The number of *E. histolytica* infections found was increased from 12 to 28 in Survey I and from 21 to 31 in Survey II. As pointed out by Sapero *et al.* (1942),

TABLE 1

Effect of the addition of penicillin and streptomycin to the medium on the growth of amebae from two types of fecal specimens

Species of amebae identified	Survey I (65 specimens)			Survey II (347 specimens)		
	Stock medium	Med. with antibiotics	Total from both media	Stock medium	Med. with antibiotics	Total from both media
E. histolytica	12(5sm)*	28(10sm)*	33(13sm)*	21(13sm)*	31(20sm)*	34(22sm)*
E. coli	1	10	11	14	36	37
E. nana	22	31	35	64	57	69
I. bütschlii	1	7	7	3	3	6
D. fragilis	1	2	2	7	14	15
Total	37	78	88	109	141	161
Percentage of total found in both media	42%	87%	100%	68%	88%	100%

* sm = small race *Eh* included in the total for the species.

small race *histolytica* often grows with difficulty. The addition of antibiotics more than doubled the number of small race *E. histolytica* infections observed in this study. The growth of both *Entamoeba coli* and *Dientamoeba fragilis* was particularly improved by use of antibiotics in both groups of specimens. Only *Endolimax nana* in Survey II grew better in the stock medium.

In addition to the increase in number of infections detected, there usually was a marked increase in the number of trophozoites in each of the cultures treated with antibiotics. This enhancement was observed in the cultures of all the species except *Iodamoeba bütschlii* and *E. nana*. The antibiotics inhibited the growth of *Blastocystis hominis* in both the original cultures and the subcultures.

Although unidentified organisms are not included in the tables, there were many trophozoites recorded as unidentified in the temporary wet mount examinations. Most of these were considered identified after examination of stained films of PVA-fixative sediments. Following these examinations there were still nine instances in which amebae seen in the permanently stained mounts could not be identified.

Comparison of cultivation with other diagnostic procedures. A comparison of the number of amebic infections found by cultivation, by sedimentation, and in stained films is shown in Table 2. In Survey I, cultivation revealed 49 per cent of the total infections found by all three techniques; FE Sed., 74 per cent; and stained film examination, 80 per cent. In Survey II, 86 per cent of 188 infections were identified in culture as compared to 49 per cent in sedimentation and 79 per cent in the stained smear. It is evident, therefore, that when the old, normally passed specimens were inoculated, cultivation was the least efficient technique used; and, when fresh, post-cathartic specimens were available, it was as efficient as the examination of stained smears and more efficient than sedimentation. The variations in the relative efficiency of the three techniques in

TABLE 2

Amebic infections found by three techniques

	Survey I (65 mailed specimens)				Survey II (347 fresh specimens)			
	Cult.	FE Sed.	Stained film	Total	Cult.	FE Sed.	Stained film	Total
E. histolytica	20	25	28	32	12	8	12	13
E. hist. (small)	13	13	19	28	22	14	24	27
E. coli	11	48	42	50	37	32	37	45
E. nana	35	33	38	47	69	37	63	79
I. bütschlii	7	14	15	19	6	2	6	9
D. fragilis	2	0	1	3	15	0	7	15
Total infections	88	133	143	179	161	93	149	188
Per cent of the total found by three techniques	49%	74%	80%	100%	86%	49%	79%	100%

detection of each species of amebae can be seen in the table. In general, the results in regard to the individual species paralleled the total results of each technique. Although not included in the table, it should be pointed out that in Survey I, where only 49 per cent of the infections were revealed by cultivation, 17 infections (9 per cent of the total) were found in the cultures that were not seen in the other examinations, and in Survey II, 32 infections (17 per cent of the total) were detected only in the cultures.

Combinations of techniques. Since it is usually necessary in a diagnostic laboratory to perform routinely a minimum number of examinations on each specimen, Table 3 was constructed to show the number of infections that were found by various combinations of two of the three techniques used. The combination of cultivation with stained films revealed 92 per cent of 179 infections in Survey

TABLE 3

Amebic infections found by various combinations of techniques

	Survey I (65 specimens)				Survey II (347 specimens)			
	Cult. and stained film	Cult. and FE sed.	Stained film and FE sed.	All 3 tech.	Cult. and stained films	Cult. and FE sed.	Stained film and FE sed.	All 3 tech.
E. histolytica	30	29	30	32	13	12	12	13
E. hist. (sm)	26	20	24	28	26	25	23	27
E. coli	43	49	49	50	43	43	43	45
E. nana	45	44	41	47	78	75	65	79
I. bütschlii	18	17	17	19	9	6	6	9
D. fragilis	3	2	1	3	15	15	7	15
All species	165	161	162	179	184	176	156	188
Per cent of total found by three techniques	92%	90%	91%	100%	98%	94%	83%	100%

I, and 98 per cent of 188 infections in Survey II. The combination of cultivation with sedimentation gave practically the same results in each survey (90 per cent and 94 per cent, respectively). Although the combination of stained film and sedimentation was just as efficient as the other two with Survey I specimens, it was significantly less efficient with the second survey specimens (83 per cent). In the detection of individual species, the combination of cultivation with stained film examination (in comparison with the other two combinations) revealed the same or an increased number of infections for all species with one exception, *E. coli* in Survey I.

DISCUSSION

From the above information, it is evident that the use of 250 units each of penicillin and streptomycin in the cultures improved the results obtained by routine cultivation of specimens from both surveys, and that, in general, growth of all the species was improved. Inasmuch as only one concentration of antibiotics was used, it can be assumed that the optimum amount to foster maximum growth of the various species was not always present in the medium. However, in the experience of the authors with LER medium, the growth of amebae is usually better when the multiplication of the bacteria is not overly inhibited by antibiotics.

In this study, cultivation as a single diagnostic technique was not efficient in comparison with the other procedures in Survey I, but was quite efficient in Survey II. Two factors may have influenced the different results obtained by cultivation in the two surveys: (a) the age of the specimens, and (b) the stage of the amebae present at time of inoculation into the medium. In Survey I, the specimens were all several days old at the time of inoculation. From what is known of the biology of intestinal amebae, it is reasonable to assume that most, if not all, of the trophozoites originally present in the normally passed stools were dead when the cultures were started (Tsuchiya, 1945). Successful establishment of cultures then can probably be attributed entirely to viable cysts which were several days old. In Survey II, the specimens were all less than 6 hours old at the time of inoculation. Moreover, many were known to have contained trophozoites (Brooke *et al.*, 1954). Since in these specimens, then, both trophozoites and cysts had an opportunity to develop, it cannot be determined definitely which factor influenced most the results obtained: age of specimen or stage of organisms present.

In evaluating cultivation as a diagnostic technique, the time involved and the difficulties encountered must be considered. In this connection, the fact that cultivation is a time-consuming procedure is pertinent. Although presumptive reports on positive specimens can sometimes be given in 24 hours, from 4 to 6 days were usually required in these surveys for a complete examination. It was calculated that the preparation of the medium and processing 30 to 60 specimens a week constituted a full-time job for one person. It should be emphasized that this routine included preparation and examination of permanently stained smears of culture sediments. Since identification of trophozoites in cultures is frequently difficult in wet mount examinations because they do not always

stain perfectly with the supra-vital stains (Quensel's may not stain at all), the examination of the stained films was often necessary for species identification. A comparison of the efficiency of the temporary wet mounts versus stained slides of cultures preserved in PVA-fixative has been analyzed in another paper (Norman and Brooke, in press).

Since the relative efficiency of the combination of two of the three techniques used differed in the two surveys (Table 3), the choice of techniques in a diagnostic laboratory should depend upon the type of specimens received. For example, it would not be advantageous in Public Health laboratories, which usually receive specimens by mail, to employ a combination including cultivation (the combination of concentration and permanently stained PVA-fixed films would be a good choice). Although, with fresh, post-cathartic specimens (Survey II), the combination of cultivation and stained film gave the best results, it would seem advisable for clinic and hospital laboratories to select another combination since both of these techniques are effective in the recovery of trophozoites but not so efficient for cysts or eggs. Therefore, in these laboratories receiving fresh specimens, we believe it would be preferable to combine cultivation (for trophozoites) and concentration (for cysts and eggs).[1]

SUMMARY

1. Four hundred and twelve fecal specimens from two surveys were used to evaluate (a) the effect of adding antibiotics to the culture medium employed in the diagnosis of intestinal amebae and (b) cultivation as a diagnostic technique in comparison with other procedures performed on the same specimen. The cultivation routine included the examination of permanently stained films prepared from culture sediments preserved in PVA-fixative. This type of examination facilitated the identification of organisms and increased the number of infections found.

2. The addition of small amounts of penicillin G sodium and streptomycin sulfate to LER medium significantly increased the number of infections of intestinal amebae found in both old, normally passed specimens (Survey I) and fresh, post-cathartic specimens (Survey II). In general, the individual species reacted similarly to the addition of the antibiotics.

3. As a diagnostic technique, cultivation was ineffective with old, normally-passed specimens, but superior to the other techniques used (concentration and stained smears) when fresh, post-cathartic specimens were examined. These results indicate that cultivation would not be a worthwhile routine technique for laboratories that usually receive mailed specimens (for example, public health laboratories), but would be advisable in clinics and hospitals. When fresh specimens are available, it is believed that a good routine consists of a cultivation technique (including stained preparations) for the identification of trophozoites and a concentration technique for the recovery of cysts.

[1] If this combination is used, a portion of each fecal specimen should be preserved in PVA-fixative for later staining if needed for more careful study or for a permanent record.

REFERENCES

BALAMUTH, W., AND WIEBOLDT, M., 1946. Action of streptomycin on *E. histolytica in vitro*. *J. Parasitol.* **32:** 10.

BALAMUTH, W., 1948. Comparative studies on cultivation and antibiotic treatment of human intestinal protozoa, *J. Parasitol.* **34:** 11.

BRADIN, J. L., AND HANSEN, E. L., 1950. Indirect *in vitro* action of antibiotics in comparison with activity of accepted amebacides, *Am. J. Trop. Med.* **30:** 27–41.

BROOKE, M. M., AND GOLDMAN, M., 1949. Polyvinyl alcohol-fixative as a preservative and adhesive for protozoa in dysenteric stools and other liquid materials, *J. Lab. Clin. Med.* **34:** 1554–1560.

BROOKE, M. M., DONALDSON, A. W., AND BROWN, E., 1954. An amebiasis survey in a Veterans Administration hospital, Chamblee, Georgia, with comparison of techniques, *Am. J. Trop. Med. & Hyg.* **3:** 615–620.

CRAIG, C. F., 1944. *The etiology, diagnosis, and treatment of amebiasis*, The Williams and Wilkins Company.

DOBELL, C., 1942. Some new methods for studying intestinal amebae and other protozoans, *Parasitology* **34:** 101–112.

FAUST, E. C., 1952. Modern criteria for the laboratory diagnosis of amebiasis, *Am. J. Trop. Med. & Hyg.* **1:** 140–145.

INOKI, S., TAKADA, S., AND NAKABAGASKI, I., 1953. New culture mediums of *E. histolytica*, *Am. J. Clin. Path.* **23:** 197–199.

LAIRD, R. L., DRINNON, V. P., AND DAVIS, A. B., 1949. Routine culture methods in diagnosing *E. histolygica*, *J. Nat. Mal. Soc.* **8:** 198–201.

MICHAEL, K. M. M., AND COORAY, G. H., 1952. A cultivation method as an aid for routine diagnosis of amebic infections in Ceylon, *Am. J. Trop. Med. & Hyg.* **1:** 543–545.

NORMAN, L., AND BROOKE, M. M., The effectiveness of the PVA-fixative technique in revealing intestinal amebae in diagnostic cultures, (In press).

PHILLIPS, BRUCE P., 1951. Measurements of direct amebicidal potential by a micro-method for screening of drugs *in vitro*, *Am. J. Trop. Med.*, **31:** 561–565.

REARDON, L. V., 1939. Cultivation without serum, *J. Parasitol.* **25:** 13.

RITCHIE, L. S., 1948. An ether sedimentation technique for routine stool examinations, *Bull. U. S. Army Med. Dept.* **8:** 326.

SAPERO, J. J., HAKANSSON, E. G., AND LOUTTET, C. M., 1942. Occurrence of two significantly distinct races of *E. histolytica*, *Am. J. Trop. Med.* **22:** 191–208.

SVENSSON, R., 1935. (Quensel's stain.) Studies on human intestinal protozoa, *Acta. Medica Scand.*, Suppl. 70. X–115 pp.

SPINGARN, C., AND EDELMAN, M., 1947. Prolongation of the viability of cultures of *E. histolytica* by the addition of streptomycin, *J. Parasitol.* **33:** 416–418.

SPINGARN, C., AND EDELMAN, M., 1948. Use of streptomycin in cultivation of *E. histolytica* from stools, *Am. J. Trop. Med.* **28:** 825–829.

SPINGARN, C., AND EDELMAN, M., 1952. Further observations on the use of streptomycin and penicillin in cultivation of *E. histolytica* from stools, *Am. J. Trop. Med. & Hyg.* **1:** 412–416.

TSUCHIYA, H., 1945. Survival time of trophozoites of *Endamoeba histolytica* and its practical significance in diagnosis, *Am. J. Trop. Med.* **25:** 277.

VELAT, C. A., WEINSTEIN, P. P., AND OTTO, G. F., 1950. A stain for rapid differentiation of trophozoites of intestinal amebae in fresh, wet preparations, *Am. J. Trop. Med.* **30:** 43–51.

YOUNG, V. M., FELSENFELD, O., SHLAES, W. H., YOSHIMURA, T., AND STEIGMANN, F., 1951. A study of laboratory methods for diagnosing *Endamoeba histolytica* and their application to 5,048 persons from the Chicago area, *Am. J. Dig. Dis.* **18:** 126–130.

THE EFFECTIVENESS OF THE PVA-FIXATIVE TECHNIQUE IN REVEALING INTESTINAL AMEBAE IN DIAGNOSTIC CULTURES

LOIS NORMAN AND M. M. BROOKE

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia

In 1949, Brooke and Goldman reported that PVA-fixative could be used as a preservative and adhesive for amebic trophozoites growing in vitro. Laird and his co-workers (1949) used PVA-fixed smears for the final identification of *Entamoeba histolytica* growing in diagnostic cultures, but felt that with experience differential diagnosis could be made without the aid of a stained mount. On the other hand, Meyer and Johnstone (1933) stated that cultured organisms must be studied in permanently stained mounts to avoid mistakes in diagnoses. They made good preparations by mixing the culture sediment on the slide with a little serum or a small amount of negative stool and fixing while wet in Schaudinn's. However, they state that such permanent preparations from culture material are difficult to prepare and in routine diagnostic work are time consuming. For several years, the examination of permanently stained films of culture sediments preserved with PVA-fixative have been included in the routine diagnostic procedure for cultivation followed in the parasitology laboratories of the Communicable Disease Center. The following report is a comparison of the results obtained on 878 fecal cultures by the examination of (a) wet mounts and (b) iron-hematoxylin stained films of sediments preserved in PVA-fixative.

MATERIALS AND METHODS

A total of 439 fecal specimens which were collected from 412 individuals in two surveys were inoculated in duplicate into modified Boeck and Drbohlav's medium without serum (LER) and were processed according to the routine described in a previous paper (Norman and Brooke, in press). Both of the microscopic examinations reported in this study were made on the sediments in the first subculture tubes usually after 48 hours incubation at 37°C. The temporary wet mounts examined on each culture included direct mounts of the sediment in the culture overlay, and mounts stained with Quensel's (Svensson, 1935) and/or V-W-O (Velat-Weinstein-Otto, 1950) temporary stains to facilitate identification of the organisms. These mounts were all examined for variable periods of time since an attempt was made to identify all the trophozoites found.

The sediment remaining in each culture tube (approximately 1 ml.) was then mixed with 2 or 3 times its volume of PVA-fixative in a small vial and allowed to settle overnight. Two or three drops of the fixed material in the bottom of the vial were then spread evenly over the center of a 1 x 3 inch slide, allowed to dry for 12 to 24 hours at 37°C., and stained by Heidenhein's iron hematoxylin method. The permanently stained films were examined microscopically for a minimum of 10 minutes each. Since the examinations of the stained films

were performed after the wet mount examinations, the presence of organisms in the first examination influenced the time spent on the stained smears.

RESULTS

There were 212 amebic infections detected in the wet mount examinations as compared to 366 infections identified in the stained smears of the same cultures (Table 1). These numbers represent 54 per cent and 94 per cent, respectively of the 391 infections found by both techniques. Of the 391 species identifications 187 (48 per cent) were found by both examinations; 179 (46 per cent) were identified in stained films, but were either not seen or were unidentified in the wet mounts; and 25 (6 per cent) were identified in the wet mounts but not in the stained smears (23 were not seen, and 2 were unidentified). There were also 38 cultures in which rare organisms were recorded as small, medium or large trophs or as "possible *Eh*," "possible *En*," etc. on the first examination. However, in the corresponding stained smears, no organisms were found which could be considered to represent these trophozoites.

In Table 1, the infections are listed according to the species of amebae which were identified. It is evident that the examination of permanently stained films increased the numbers identified of all the species as compared to the results obtained by examination of the temporary mounts. This increase was particularly marked with *Iodamoeba bütschlii* for all 14 infections were identified only after the examination of the stained smears. The numbers of *Endolimax nana* and small race *Entamoeba histolytica* infections found in the permanent mounts were almost double those seen in the temporary mounts, while the numbers of *Dientamoeba fragilis*, *Amoeba coli*, and large race *E. histolytica* were increased to less degrees in the order listed.

When the first examinations (wet mounts) were completed, there were 97 cultures in which unidentified organisms had been seen. Only 9 of the perma-

TABLE 1

Positives resulting from examination of wet mounts and stained smear from 878 cultures

Organisms identified	Results of wet mount exams	Results of permanent stain exams (PVA-fixative)	Total by both exams
Entamoeba histolytica	40	45	50
E. histolytica small	24	48	54
Amoeba coli	50	61	67
Endolimax nana	81	174	179
Iodamoeba bütschlii	0	14	14
Dientamoeba fragilis	17	24	27
Total	212	366	391
Per cent of positives	54	94	100
Numbers of cultures containing unidentified organisms	97	9	40

nently stained films showed trophozoites which could not be identified. After the second examination (stained mounts) there were still unidentified organisms recorded in 40 cultures. In most of these cultures, only a very rare organism (or possible organism) had been seen in the wet mounts.

DISCUSSION

In comparison with the usual practice of examining only wet mounts of cultures, use of the PVA-fixative technique increased the number of identified organisms by 80 per cent (212 to 391). All of the films adhered to the slides during staining and, in general, the diagnostic features of the amebae were well preserved. Stained smears were particularly helpful with *E. nana* and small race *E. histolytica*, which are often troublesome because of their size (especially in cultures containing *Blastocystis hominis*) and because, with temporary staining, nuclei of *E. nana* sometimes appeared as a ring, suggesting *E. histolytica*. Wet mount identification of *I. bütschlii* was also difficult in this study because very few organisms were present and, when encountered, they did not always stain sharply with supravital stains. Identification of *E. coli*, especially in mixed infections with *E. histolytica*, also was often dependent upon examination of the permanently stained films. The infections missed in the stained preparations were almost always in cultures growing so poorly that only rare organisms were seen in the wet mounts.

A cultivation procedure which includes examination of both wet mounts and permanently stained preparations is time consuming, and the question arises whether the wet mount examination could not be eliminated entirely. The findings suggest that the examination of only permanently stained films might be quite efficient; however, the results of this study do not accurately represent the efficiency of either method alone, since they were not used independently. In field surveys, the examination of only PVA-fixed culture material would be of particular advantage since more specimens could be processed in a day if microscopic work could be postponed.

Another practical simplification of the culture procedure might be accomplished by examining the wet mounts of all cultures, then staining permanently only those in which trophozoites were shown to be present. Of the 878 cultures in this study, 250 were positive by wet mount examination. If the stained films of only these 250 had been examined, 368 infections would have been reported, or 94 per cent of the 391 infections found by the complete examination. Examination of the stained films of the remaining 628 cultures added only 23 infections.

The authors believe as a result of this investigation that, if cultivation is employed as a diagnostic procedure for intestinal amebae, permanently stained films by the PVA-fixative technique should be prepared from culture sediments of at least those in which organisms are observed on wet mount examination.

SUMMARY

Examinations of wet mounts and iron hematoxylin stained sediments preserved in PVA-fixative from 878 diagnostic fecal cultures were made, and the

results compared. Of a total of 391 infections found by the two examinations, 366 infections (or 94 per cent) were identified in the stained films as compared to 212 (or 56 per cent) identified in the wet mounts of the same cultures. The increased number was due (a) to the addition of infections found only in the stained films and (b) to the identification of infections seen but not identified in the wet mounts. The numbers of infections of all five species of amebae were increased to varying degrees.

The sediment from routine diagnostic fecal cultures for amebae can be fixed with PVA-fixative and stained by permanent staining techniques with good results. In order to reduce the work involved in the cultivation procedure, it is suggested that the preparation of stained smears be reserved for those cultures in which organisms are observed in wet mounts. In this study 94 per cent of the infections would have been identified by this limited number of examinations of stained slides (250 slides rather than 878).

REFERENCES

BROOKE, M. M., AND GOLDMAN, M., 1949. Polyvinyl alcohol fixative as a preservative and adhesive for protozoa in dysenteric stools and other liquid materials, *J. Lab. Clin. Med.* **34**: 1554–1560.

LAIRD, R. L., DRINNON, V. P., AND DAVIS, A. B., 1949. Routine culture methods in diagnosing *E. histolytica*, *J. Nat. Mal. Soc.* **8**: 198–201.

MEYER, K. F., AND JOHNSTONE, H. G., 1935. Laboratory diagnosis of amebiasis, *Am. J. Pub. Health* **25**: 405–414.

NORMAN, LOIS, AND BROOKE, M. M. The use of penicillin and streptomycin in the routine cultivation of amebae from fecal specimens, In press.

SVENSSON, R., 1935. (Quensel's stain). Studies on human intestinal protozoa, *Acta Medica Scand.*, Suppl. 70. X–115 pp.

VELAT, C. A., WEINSTEIN, P. P., AND OTTO, G. F., 1950. A stain for rapid differentiation of trophozoites of intestinal amebae in fresh, wet preparations, *Am. J. Trop. Med.* **30**: 43–51.

HISTORICAL EVIDENCE FOR INTERFERENCE BETWEEN DENGUE AND YELLOW FEVER

HARALD FREDERIKSEN, M.D., M.P.H., D.T.M. & H.

"Just as one nail drives out another, so does epidemic drive out epidemic. I do not mean that the disease which is expelled and which retreats, disappears altogether. I only mean that it decreases in frequency." (Sydenham, 1670)

Recent geographic and laboratory evidence suggests the possibility of such reciprocal behavior for dengue and yellow fever. May, in his study of the world distribution of dengue and yellow fever, found that dengue is most heavily reported where yellow fever is unknown. Dengue is common in Asia and the South Pacific. May describes as the most striking among all the geographical and epidemiological features of yellow fever the total absence of yellow fever in Asia and the South Pacific, in regions where several of the known vectors are abundant and where men live in ecological conditions most suitable for the dissemination of the disease (May, 1952).

Dudley finds it so remarkable that yellow fever has never reached Asia by the Mediterranean route as to make one suspect that there must be some reason other than lack of suitable mechanical transport for the virus; it almost justifies the assumption that Asia can only have remained free from yellow fever up to the present because of some biological obstruction to the free spread of virus on its arrival there (Dudley, 1934). It might be mentioned that Hindle was able to prove that the Indian race of *A. aegypti* is able to transmit the yellow fever virus from monkey to monkey in the laboratory (Hindle, 1929).

Similar considerations led Sabin to study interference between dengue and the viscerotropic yellow fever virus in rhesus monkeys and mosquitoes (Sabin, 1952). Sabin states: "One of the reasons the problem of interference between these two viruses was pursued further is the peculiarity in the epidemiology of yellow fever that has apparently spared many parts of the world (e.g., India, Indonesia, Australia, etc.) where dengue has been endemic.

"When dengue virus was injected in rhesus monkeys 2–3 days before the viscerotropic yellow fever virus (Asibi strain), it interfered with the multiplication of the latter virus and 6 of the 7 monkeys survived while all 9 control monkeys inoculated with yellow fever died. When the yellow fever virus (100 M.L.D.) was injected one month after the dengue virus, 6 of 8 monkeys died in a manner which suggested that no significant cross immunity existed between the two viruses.

"Since the *Aedes aegypti* mosquitoes serve as natural vectors for both dengue and yellow fever viruses and since the available evidence indicated that mosquitoes remain infected for life the possible occurrence of interference between these two viruses in mosquitoes was of special interest and possible epidemiologic significance.

"Experiments were performed in which normal and dengue infected mosquitoes were allowed to feed on artificial mixtures containing varying amounts of yellow

fever virus, and the multiplication of virus in them determined by tests in mice. These experiments strongly suggested interference with multiplication of smaller doses (about 10^5 LD_{50} or less) of yellow fever in dengue infected mosquitoes. In another biting test in monkeys, the normal mosquitoes which ingested 10^5 LD_{50} of virus transmitted yellow fever, while a similar number of dengue infected mosquitoes which fed on the same mixture did not.

"These results suggest the possibility that the introduction of yellow fever virus in a dengue-endemic area may find enough mosquitoes relatively refractory to the yellow fever virus to prevent the establishment of yellow fever in the same area." (Sabin, 1952)

If the interference phenomenon plays any role in the distribution of these viruses, it presumably becomes operative in the mosquito which remains infected for life, whereas in man or reservoir the interference phenomenon is only of a few days duration. In the laboratory the mosquitoes infected with dengue virus were found to be partially refractory to yellow fever virus; however, this makes one speculate on the percentage of mosquitoes infected with dengue in epidemic and endemic situations. Unfortunately very little is known about the percentage of mosquitoes carrying the virus during epidemics.

The geographical evidence for the reciprocal behavior of dengue and yellow fever and the experimental findings of interference in the vector are very suggestive; however, it appears that additional epidemiological studies are needed to determine the importance of the interference phenomenon in nature. This suggested the study of the history of dengue and yellow fever epidemics, particularly before the days of Reed, when yellow fever and dengue were not restricted in their distribution.

The earliest description of dengue may be the *coup de barre* or *fiebre amarilla frusta* which reigned in the Lesser Antilles during the intervals between the epidemic periods of true yellow fever and which afflicted the French colonists from their earliest occupation of those islands, in the 17th century (Siler, 1926). However, dengue has first been recognized with certainty and frequency in areas where yellow fever was known to occur during the 19th century.

During the 19th century two great epidemics of dengue swept the Southern parts of the U. S., in 1828 and 1850 (Hirsch 1883). Yellow fever epidemics occurred yearly in this area. Between 1820 and 1860 the U. S. Marine Hospital Service records 172 outbreaks of yellow fever in U. S. seaports (USMHS, 1895). This situation seemed to prevent an ideal opportunity to examine the possibility of interference or free co-existence of dengue with yellow fever.

The situations, when dengue and yellow fever may have coexisted, seem to be few and exceptional. According to the available records it appears that only a few of the numerous epidemics of dengue and yellow fever occurred at the same place and during the same year. After reference to the original reports it appears that these situations do not establish the free coexistence of dengue and yellow fever but rather a tendency towards mutual exclusiveness. All of these instances are briefly presented below.

At Havana, in 1828, Osgood reports that during the winter and spring many of the late arrivals were seized with yellow fever (Osgood, 1828). Late in April,

1828, Havana was generally and almost simultaneously attacked by dengue (Osgood, 1828; Lehman, 1828). Osgood states that as soon as dengue began to prevail, yellow fever disappeared.

In Martinique, dengue was epidemic during the first months of 1828, while the yellow fever epidemic took place during the last months of 1828 and January, 1829 (Moreau de Jonnes).

In Charleston, dengue affected the greater part of the population in June and July, 1828. Upon termination of the dengue epidemic an outbreak of yellow fever took place in August and September, 1828, causing 26 deaths (Dickson, 1828, 1839, 1852; Dowler, 1859; Augustin, 1909).

In New Orleans, both dengue and yellow fever were reported in 1828. Dengue reached its peak in June (Dumarescq, 1828). However, only 12 cases of yellow fever had been admitted to the Charity Hospital by the 25th of July (Drake, 1854). Subsequently, 290 yellow fever admissions and 130 deaths were reported for the Charity Hospital in 1828 (Jones, 1890). It appears that the epidemic of yellow fever began upon the termination of the dengue epidemic.

Waring had recorded epidemics of dengue at Savannah in 1826 and 1828 in which years yellow fever was absent (Waring, 1830). For 1827, Waring reported sporadic cases of yellow fever and dengue, with a total of about 50 cases for both. Waring wrote: "Thus it appears from the history here given that since the year 1826 to this time we have been visited by a series of epidemics and other diseases tending to the periphery of the body. For a number of previous years eruptions were very rarely epidemic; we were subject to bilious and yellow fever of all grades during spring, summer and fall or nine months out of twelve."

From the reports on the epidemic at Vicksburg in 1847 it might be concluded that an incipient outbreak of yellow fever was soon followed by an epidemic of dengue. It appears that the outbreak of yellow fever was restricted both in time and place (Magruder, 1848; Hicks, 1848). The first case of yellow fever was reported on the 31st of August. At this time 8–10 cases were confined to one square in the upper portion of the city. These cases, occurring during the first week of the epidemic, died. Subsequently the disease was mild in type, characterized by an eruption consistent with the clinical picture of dengue. The eruption was not noted in malignant cases. The note that the eruption never occurred in malignant cases suggests that no persons were observed to be afflicted with yellow fever and dengue simultaneously.

Although only a sketchy description of the epidemic at Houston in 1848 is available, it resembles the pattern of dengue and yellow fever at Vicksburg in 1847 (McCraven, 1849). Six to seven cases residing at the same boarding house near the steamboat landing developed yellow fever in the last part of August and subsequently died. During the early part of September, an epidemic became general throughout the town with mild cases greatly predominating. These cases were considered to be dengue which is supported by the fact that many were attacked who had had yellow fever in previous years. During the latter part of the epidemic none of the cases were fatal.

Situations are found when dengue was immediately followed by yellow fever in circumstances unusually favorable for yellow fever. This was the case in

Natchez and New Orleans in 1848 when thousands of veterans were returning from Mexico where yellow fever was epidemic. Stone reported an epidemic of dengue characterized by severe pains and various eruptions at Natchez in 1848 (Stone, 1849). Three thousand persons among a population of 4000–5000 were affected. The peak of the epidemic was reached in August. In the beginning of the epidemic the cases recovered quickly; however, toward the end of the epidemic fatal cases were observed. A total of 42 deaths was attributed to yellow fever.

Fenner reports a similar epidemic at New Orleans in 1848 (Fenner, 1849; Am. Med. Intelligence, 1848). Fenner states the predominant fever of the summer and autumn was a mild epidemic of yellow fever. Many cases were accompanied or followed by boils and an extraordinary degree of pains in the back or limbs which gave rise to the opinion that dengue was prevailing with yellow fever.

Assuming that some of these cases were dengue, no information on the numbers or months of occurrence could be found. However, admissions and deaths due to yellow fever listed in Table 1, indicate that the fatality rate for yellow fever had increased several times from August to October (Jones, 1890; Fenner, 1849). This suggests that the dengue epidemic preceded the yellow fever epidemic, and the nonfatal dengue was confused with yellow fever, erroneously lowering the fatality rate of yellow fever during August. It appears that the mild disease of the summer may well have been dengue, whereas the figures presented in Table 1 indicate that yellow fever took place in fall, which corresponds to the happenings at nearby Natchez in 1848.

It may seem that dengue did not materially reduce the incidence of yellow fever in 1848. However, it is noted that yellow fever was unusually favored this year. Between May and August 30,000 troops returned to New Orleans from Mexico where yellow fever was epidemic. Cases of yellow fever were noted on board the ships returning the troops in groups of 150–450. Considering this extraordinarily massive and continuous introduction of yellow fever, the 420 yellow fever deaths at the Charity Hospital in 1848 are put in perspective by the 895 deaths of the preceding year and 545 deaths of the following year at the Charity Hospital.

Hirsch reports dengue and yellow fever in New Orleans also during 1849 (Hirsch, 1883). However, journals and official reports from New Orleans failed to mention a dengue epidemic in 1849.

Particularly striking is the virtual absence of yellow fever from the U. S. seaboard co-incident with the dengue pandemic in 1850. New Orleans was the

TABLE 1

Yellow fever deaths at New Orleans, yellow fever admissions and deaths at the Charity Hospital, New Orleans, 1848. (Jones, 1890; Fenner 1849)

	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Deaths, New Orleans		4	33	220	467	226	20		808
Admissions, Charity Hospital	2	2	31	462	597	105	34	1	1234
Deaths, Charity Hosp.		2	11	?	209	70	?		420

TABLE 2

Yellow fever deaths in U. S. ports (U. S. Marine Hospital Service, 1895, supplemented with data from Augustin, 1909)*

Year	Galveston	New Orleans	Mobile	Key West	Pensacola	Savannah	Charleston	Norfolk	Portsmouth	Philadelphia	New York
1846		146	4		?						
1847	200	2259	78		?						
1848		850	24		?						12
1849		737	21				125				
1850		102									
1851		16									
1852		415				19	310	3*	4*		1
1853	536	7910	1191*	112	260*	?		1600		128	14
1854	404	2423		98*	?	580	627	?	2*	?	20
1855		2670						1807	1000		5

? Deaths not listed.

* Augustin 1909.

only city in the U. S. visited by yellow fever in 1850. This was rather unusual considering the experience of previous and subsequent years illustrated by Table 2, (U.S.M.H.S., 1895; Augustin, 1909).

A large part of the population of New Orleans was attacked by dengue, which prevailed throughout the summer of 1850 (City Medical Intelligence, 1850). The unusually small number of yellow fever cases in New Orleans during 1850 may be significant. The first case of yellow fever was admitted to the Charity Hospital on August 23. During this year a total of 10 yellow fever cases and only four yellow fever deaths were reported for the Charity Hospital at New Orleans (Jones, 1890). The deaths due to yellow fever in 1850 are placed in perspective when compared with the deaths for the years 1846–1885 listed in Table 3 (Jones, 1890).

Charleston was universally attacked by dengue in 1850 and again invaded by dengue in 1851 during which years no deaths were attributed to yellow fever, whereas Charleston had 125 yellow fever deaths in 1849 and 310 such deaths in 1852 (Dickson, 1852 U.S.M.H.S., 1895).

Hume was struck by the absence of yellow fever in the presence of dengue at Charleston which he discusses as follows: "To return to 1850: according to conclusions drawn from preceding years, yellow fever should have prevailed, but it did not; that a malarial atmosphere existed is evident from the prevalence of breakbone fever, but there was yet wanting some element or condition to produce the yellow fever. If we admit the substitution of one for the other, or the prevalence of one disease excluding another, the summer of 1850 will take its place with 1849 and 1852 as its meteorological phenomena indicate and the correctness of our conclusions will not be impaired." (Hume, 1853).

However, Hume points out that his speculations seem contrary to the truth for dengue and yellow fever both prevailed at Charleston in 1852. La Roche also reports that dengue accompanied the yellow fever epidemic in 1852 (La Roche, 1855). Unfortunately, these authors neither describe the cases nor indicate the

TABLE 3

Yellow fever deaths at the Charity Hospital, New Orleans 1846–1855

Year	*Deaths*
1846	96
1847	895
1848	420
1849	545
1850	4
1851	2
1852	339
1853	1890
1854	1233
1855	51

extent and nor months of occurrence of the dengue epidemic. Fortunately, Porter gives a detailed description of the epidemic situation at Fort Moultrie on Sullivans Island in the harbor of Charleston in 1852 (Porter, 1854). Porter describes an epidemic resembling dengue taking place in September. He lists his cases as bilious remittent fever, a term he had previously used to describe the dengue epidemic of 1850. (Dickson, 1852). Yellow fever took place on Sullivans Island during October and November which seems to reflect the situation on the mainland where yellow fever reached its peak in October (Dowler, 1859).

The abstract of the quarterly reports of Fort Moultrie listed in Table 4 gives a striking picture of the situation (Porter, 1854). It might be noted that Porter's detailed clinical descriptions give the assurance that this picture is not the result of erroneous reporting. The cases in September are consistently described as mild, characterized by frontal, orbital, back and limb pains, whereas most cases of October and November, in addition to these complaints, experienced jaundice or hemorrhages, four of these cases terminating fatally.

If the cases of remittent fever are accepted as dengue, then the Quarterly Reports give another example of the distinct occurrence of dengue and yellow fever, the former in September the latter in October and November. On the other hand, if the cases of remittent fever are not accepted as dengue, then little or no evidence could be found to confirm the existence of dengue at Charleston in 1852. In either event the speculations of Hume that the prevalence of dengue may have excluded yellow fever in 1850 remain unimpaired.

Mobile was the only Southern city where a dengue epidemic was reported in 1854 (Hirsch 1883). On inspection of Table 2 it is interesting to note that Mobile was spared by yellow fever in 1854 when many Southern cities were ravaged by severe epidemics of yellow fever. Although Hirsch mentioned yellow fever at Mobile in 1854, Augustin reports only sporadic cases of yellow fever and lists no deaths for this year (Augustin, 1909).

Hirsch also lists both dengue and yellow fever epidemics for Martinique in 1860 (Hirsch, 1883). While the report of Ballot gives an unmistakable description of a dengue epidemic from June until December, 1860, there is no indication of yellow fever occurring at this time (Ballot, 1870).

At this point it is interesting to present one situation where there is little

TABLE 4

Remittent and yellow fever at Fort Moultrie, Charleston 1852

	September		October		November	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
Yellow Fever	1	0	29	3	3	1
Remittent Fever	24	0	3	0	1	0

doubt that dengue and yellow fever affected one group simultaneously, although in somewhat unusual circumstances. This outbreak which took place on the H.M.S. Scout in 1866 is described by Dudley as follows:

"The H.M.S. Scout in 1866 was infected with typical yellow fever at the Island of Taboga just south of Panama; there being no doubt of the diagnosis, the Scout fled north. The ship's surgeon was one of the first fatal cases, so the Assistant Surgeon, Carmichael, took medical charge. This officer relates how, after the ship had put to sea, the character of some of the cases underwent a peculiar change. Dragging pains in the loins and general muscular pains were the most prominent symptoms; yet, strange to relate, these cases were in other respects the mildest and the patients had least signs of kidney trouble. They were, however, subject to irregular raised scarlatiniform eruptions, followed by desquamation of the palms of the hands and soles of the feet. All this time malignant and fatal cases of yellow fever with black vomit and albuminuria continued to crop up. Carmichael says that the symptoms of these milder cases bore such a close resemblance to those of broken back fever—the dengue of American authors—as to suggest the idea of their identity. He, however, decided that the existence of the two diseases at the same time was so improbable that all his 36 cases must have been yellow fever. The account is so lucid and circumstantial that there can be no reasonable doubt that the mosquitoes on board the Scout were infected with the viruses of both diseases." (Dudley, 1932–33)

Although this is a situation where dengue and yellow fever affected one population at one time, this instance does not quite meet the criteria of co-existence at the same time and place in view of the mobility of the population. Nothing is known about the course of the ship, nor which epidemics ravaged the ports of call. On the basis of this report, one cannot assume that both diseases co-existed in any one port; rather one might assume that it was possible for the ship to be infected with yellow fever in one port and dengue in another, whereby the cases on the ship might be considered dependent on the happenings in different ports. It might be observed that eruptions were not noted in the severe or fatal cases. There is no evidence that any of the crew or mosquitoes were infected with both viruses simultaneously.

Cazanove reviewed the history of dengue and yellow fever epidemics in Senegal and uncovered several occasions where dengue preceded yellow fever (Cazanove, 1930). He was particularly struck by the fact that dengue and yellow fever in Senegal were never exactly superimposed. Cazanove reports a dengue epidemic at Goree, Senegal in June, 1878, and a yellow fever epidemic

which took place two months later. Augustin also refers to cases of dengue occurring during the first half of 1878 and a yellow fever epidemic during the latter half of 1878, the first yellow fever death occurring on July 13 (Augustin, 1909).

A number of authors describe dengue epidemics in various localities of Senegal during the period from July until October, 1926, whereas yellow fever, aside from two cases in July, did not occur until the termination of the dengue epidemic in October. Ramsey reports that isolated cases of yellow fever began to be reported in October and for the remainder of the year. The following year yellow fever was epidemic in Senegal (Ramsey, 1931). This situation seems most interesting. The report of 2 cases of yellow fever in July indicates that the virus was present. The dengue epidemic suggests that the vector was in abundance and the occurrence of yellow fever at the end of 1926 and during the following year indicates that susceptibles to yellow fever were also present. Although all factors considered necessary for the maintenance of an epidemic seemed to be present during the summer of 1926, the outbreak of yellow fever first took place in October upon the termination of the dengue epidemic.

As a final point, attention should be given to jungle yellow fever which was discovered when the hopes for the eradication of yellow fever seemed so near fulfillment. Jungle yellow fever has now been found to exist in many parts of Africa, Central and South America. Conversely, only little is known about jungle dengue fever. However, Simmons found that the monkeys are probably an important factor in the spread of dengue in the Philippines (Simmons *et al.*, 1931). This suggests that jungle dengue fever may be as important in the epidemiology of human dengue fever as jungle yellow fever is in the epidemiology of human yellow fever and indicates the need for study of the relationships of dengue and yellow fever in their jungle ecology. These data suggest additional experimental and epidemiological studies to evaluate the remote possibility of applying the interference phenomenon to the control of disease in situations not amenable to other methods of control, such as jungle yellow fever.

SUMMARY

Reviewing the history of the epidemics of dengue and yellow fever, little or no evidence can be found that these diseases coexist freely. In their history dengue and yellow fever seemed to display a tendency toward mutual exclusiveness, consistent with the geographical and experimental evidence.

REFERENCES

Am. Med. Intelligence, 1848. *New Orleans Med. and Surg. J.* **5**: 397.

Augustin, G., 1909. *History of Yellow Fever*. New Orleans. Searcy and Pfaff.

Ballot, 1870. Epidemie de dengue a la Martinique en 1860, *Arch. Med. Nav.* **13**: 470–473.

Cartron, A., 1929. Note on an epidemic of dengue at St. Louis, Senegal in 1926, *Bull. Soc. Path. Exot.* **22**: 228–229.

Cazanove, F., 1930. Dengue et fievre jaune, *Bull. Soc. Path. Exot.* **23**: 155–161.

City Med. Intelligence, 1850. *New Orleans Med. and Surg. J.* **7**: 401–403.

Dickson, H. S., 1828. Account of the Dengue as it appeared in Charleston, S. C. during the Summer of 1828, *Am. J. Med. Sc.* **3**: 3–14.

Dickson, H. S., 1839. *On Dengue, Its History, Pathology and Treatment*. Philadelphia. Haswell, Barrington and Haswell.

DICKSON, H. S., 1852. On the blending and conversion of types of fever, *Transactions of the A.M.A.* **5**: 127–161.

DOWLER, B., 1859. Yellow fever of Charleston, 1858. *New Orleans Med. & Surg. J.* **16**: 596–600.

DRAKE, D., 1854. *Treatise on the Principal Diseases of the Interior Valley of North America.* Cincinnati.

DUDLEY, S. F., 1932–33. Yellow fever as seen by the Medical Officers of the Royal Navy in the 19th century, *Proc. Royal Soc. Med.* **26**: 443–456.

DUDLEY, S. F., 1934. Can yellow fever spread into Asia? An essay on the ecology of mosquito borne disease, *J. Trop. Med. & Hyg.* **37**: 273–278.

DUMARESCQ, P. J., 1828. *Boston Med. & Surg. J.* **1**: 497.

FENNER, E. D., 1849. An account of the yellow fever at New Orleans in the year 1848, *New Orleans Med. and Surg. J.* **6**: 2–52.

HICKS, B. J., 1848. On the yellow fever of Vicksburg, Miss. in the year 1847, *New Orleans Med. & Surg. J.* **6**: 220–225.

HINDLE, E., 1929. *Transactions Royal Soc. Trop. Med. and Hyg.* **12**: 405.

HIRSCH, A., 1883. *Handbook of Geographical and Historical Pathology.* New Sydenham Society. London.

HUME, W., 1853. Sequel to meteorological and other observations in reference to the causes of yellow fever in Charleston, brought forward to 1852, *Charleston Med. J. and Rev.* **8**: 55–67.

JONES, J., 1890. Medical and Surgical memoirs containing investigations on the geographical distribution, causes, nature, relations and treatment of various diseases. **3**: part 1. New Orleans.

LA ROCHE, R., 1855. *Yellow Fever, considered in its historical pathological, etiological and therapeutical relations.* **1**: 444, Philadelphia. Blanchard and Lea.

LEHMAN, G. F., 1828. Account of the disease called dengue which has prevailed so extensively at Havana, *Am. J. Med. Sc.* **2**: 477.

MAGRUDER, A. L. C., 1848. A history of the epidemic which prevailed in Vicksburg during the fall of 1847, *New Orleans Med. & Surg. J.* **4**: 689–697.

MAY, J. M., 1952. Map of the world distribution of dengue and yellow fever, *Geographical Review* **42**: 283–292.

MCCRAVEN, W., 1849. Letter on yellow fever, *New Orleans Med. & Surg. J.* **6**: 60–64.

MOREAU DE JONNES. Epidemies des antilles en 1828, *J. Gen. de Med.* **108**: 99.

OSGOOD, D., 1828. Remarks on dengue, *Boston Med. & Surg. J.* **1**: 561–563.

PERVES, 1927. Epidemie de dengue a la marine de Dakar, *Bull. Soc. Path. Exot.* **20**: 92–99.

PORTER, J. B., 1854. On the climate and salubrity at Fort Moultrie and Sullivan's Island, Charleston Harbor, S. C. with incidental remarks on the yellow fever of the City of Charleston, *Am. J. Med. Sc.* **28**: 21–75.

RAMSEY, G. H., 1931. Yellow fever in Senegal with special reference to 1926 and 1927 epidemics, *Am. J. Hyg.* **13**: 129–163.

SABIN, A., 1952. Research on dengue during World War II, *Am. J. Trop. Med. & Hyg.* **1**: 30–50.

SILER, J. F., HALL, M. W., AND HITCHENS, A. P., 1926. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity and prevention, *Philippine J. of Sc.* **29**: 1–304.

SIMMONS, J. S., ST. JOHN, J. H., AND REYNOLDS, F. H. K., 1931. Experimental studies of dengue, *Philippine J. Sci.* **44**: 1–247 (Monograph 29 of the Bureau of Science, Manila.)

STONE, C. H., 1849. History of the mild yellow fever which prevailed epidemically in the City of Natchez during a part of the summer and fall of 1848, with observations respecting its character and natural mode of cure, *New Orleans Med. & Surg. J.* **5**: 570–593.

SYDENHAM, T., 1670. *Constitution of Epidemics.* 1–2. Sydenham Society, London. 1848.

U. S. Marine Hosp. Service, 1895, *Annual Report*, 428–439.

WARING, W. R., 1830. A summary of the climate and epidemics of Savannah during the series of years from 1826 to 1829, *Am. Med. & Surg. J.* **9**: 374–392; **10**: 136–145.

CLASSIFICATION OF LEPTOSPIRAL ISOLATES FROM MALAYA, THAILAND AND NORTH BORNEO

AARON D. ALEXANDER, PSYCHE W. WETMORE, LARUE B. EVANS, HELEN JEFFRIES AND CHESTER A. GLEISER

Veterinary Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

The significance of Southeast Asia as an important focus of epidemic and endemic leptospirosis in man and animals was recognized mainly as the result of intensive investigations by Dutch workers in Indonesia (Walch-Sorgdrager, 1939). Leptospiral research in the Indonesian Archipelago, initiated by Schüffner in 1918 and conducted up to the outbreak of World War II, contributed in large measure to our current concepts of leptospirosis. It is interesting to note that 18 of the 34 currently recognized distinct antigenic strains of leptospires were initially recognized in Indonesia (Collier, 1948).

Based upon stable and specific antigenic characteristics as revealed by the agglutination-lysis reaction, members of the genus *Leptospira* have been classified into serogroups, comprised of serotypes, sharing major antigenic components (Wolff and Broom, 1954). By definition, a serotype includes all strains indistinguishable one from the other on the basis of cross-absorption procedures. Within the serogroup, in addition to heterologous serotypes, "complete" and "incomplete" biotypes may be found. The "incomplete" biotype contains part, but not all of the antigenic components of the "complete" biotype. Wolff and Broom's currently proposed classification of leptospires groups 34 leptospiral serotypes into 20 serogroups. This scheme has been tentatively accepted by various leptospiral typing laboratories as a working basis in the typing of leptospiral strains.

Outside of Indonesia, there have been limited precise serologic identifications of leptospiral isolates from other sections of Southeast Asia. In Malaya the early studies of Fletcher (1928) on 26 human isolates disclosed six distinct antigenic types, three of which were related to *L. pyrogenes*, *L. autumnalis* and *L. icterohemorrhagiae*, respectively, and three of which could not be identified with the type strains then available. In addition, he isolated strains from black rats and a dog which were related to *L. autumnalis*. Subsequent to Fletcher's studies, interest in leptospirosis in Malaya waned until the recent outbreaks of banditry in this area, and the consequent operations of British troops in jungle areas elicited a high incidence of this disease (Broom, 1953; Hughes, discussion following Broom's paper; Ley *et al.*, to be published). In Broom's (1953) recent study *L. bataviae* (3 strains), *L. canicola* and *L. bangkinang* were identified in five human isolates, and he obtained serological evidence of a multiplicity of serotype strains infecting humans.

There is a paucity of information on the prevailing leptospiral serotypes found in North Borneo and Thailand. *L. bataviae* is reportedly present in South Borneo (Lingen, 1933), and Sundharagiati and Buspavanich (1951) recently iden-

tified strains isolated from humans as *L. bataviae* and a member of the serogroup *autumnalis*, closely related to *L. rachmat*.

During the course of an epidemiologic survey of leptospirosis in Malaya, North Borneo and to a limited extent Thailand, conducted by the Army Medical Service Graduate School (Wisseman *et al.*, 1954) leptospiral isolates were obtained from members of British security forces hospitalized in Kuala Lumpur, and from various wild rodents trapped in Malaya, Thailand and North Borneo. The authors report the results of an antigenic analysis of these leptospires based upon the agglutination-lysis technique.

MATERIALS AND METHODS

Strains studied. Twenty leptospiral strains isolated from eight humans and twelve rodents were studied. The source and designation of the isolated strains are shown in Table 1. All of the human isolates were obtained from direct blood cultures in appropriate leptospiral mediums except for strain H-3 which was isolated from the blood of a hamster inoculated with the patient's blood. The techniques employed in the isolation of the leptospiral strains have been described in previous reports (Wisseman *et al.*, 1954; Gochenour *et al.*, 1953).

Preparation of immune serums. Normal rabbits weighing approximately three kilograms were inoculated with 1.0 ml., 2.0 ml., 4.0 ml., and 6.0 ml. of a five to seven-day-old live Fletcher's (1928) culture of the desired leptospiral strains at five to seven-day intervals. Five to seven days following the last inoculation, the rabbits were exsanguinated and the serums harvested. The serums, after Seitz filtration, were stored at −20°C.

Agglutination-lysis test. Serial 4-fold dilutions were prepared with each serum employed in the test, using buffered saline (pH 7.4) as diluent and providing serum dilutions of from 1:50 through 1:204,800. To 0.2 ml. of each serum dilution in a standard Kahn tube was added an equal quantity of antigen, which consisted of five to seven-day-old culture in Schüffner's medium (Gochenour, 1953). The tubes were shaken, incubated at 30°C. for three hours and then examined by (10 × objective and 15 × ocular) dark-ground illumination without the use of cover glasses. Agglutination or lysis, or both, were read as complete, partial, trace or absent. Titers were expressed as the reciprocals of the highest dilution in which complete agglutination or lysis was observed.

Absorption procedure. The antigenic analysis of the isolated strains was carried out over a period of approximately two years, during which time the original agglutinin-absorption technique was modified. Consequently some of the strains were studied by the original techniques; however the basic findings in these studies were rechecked employing the modified technique and no significant differences in findings were evidenced. Both techniques are described.

Agglutinin-absorption procedure I. Absorbing antigen was prepared from seven to nine-day-old leptospiral cultures maintained in Stuart's (1946) medium at 30°C. Cultures were centrifuged at approximately 4000 g. in the International Centrifuge PR-1 for one hour in 100 ml. cups employing International Centrifuge head #845. The supernatant fluid was discarded and the leptospires were

TABLE 1

Source of leptospiral strains studied

STRAIN	SOURCE		DESIGNATION[a]
	Species	Place	
GB-387	Human	Malaya	H - 1
GB-13	Human	Malaya	H - 2
561	Human	Malaya	H - 3
B-1922	Human	Malaya	H - 4
GB-462	Human	Malaya	H - 5
GB-441	Human	Malaya	H - 6
GB-511	Human	Malaya	H - 7
GB-438	Human	Malaya	H - 8
ML - 2	*Rattus rajah*	Malaya	RR - 1
B-8699	*Rattus mullerei*	Malaya	RM - 2
ML-19	*Rattus mullerei*	Malaya	RM - 3
B-8698	*Rattus mullerei*	Malaya	RM - 4
B-8697	*Rattus sabanus*	Malaya	RS - 5
B-14083	*Rattus whiteheadi*[b]	North Borneo	RW - 6
RTB-10336	*Bandicoota bengalensis*	Thailand	BB - 7
RTB-14001	*B. bengalensis*	Thailand	BB - 8
RTB-14006	*B. bengalensis*	Thailand	BB - 9
RTB-14007	*B. bengalensis*	Thailand	BB - 10
RTB-14020	*B. bengalensis*	Thailand	BB - 11
RTB-10416-18	*B. bengalensis*	Thailand	BB - 12

[a] Denotes designation used in this paper by the author.

[b] Rodent was trapped in Tenompak, Mt. Kinabalu, elevation 4500 feet.

resuspended to approximately 1% of the original culture volume with buffered saline (pH 7.4). Four parts of the absorbing antigen were added to one part of a 1:10 dilution of immune serum, placed in a shaker apparatus for two hours and maintained overnight in a 30°C. incubator. The absorbing mixture was then centrifuged at approximately 20,000 g. for ten minutes in the Inter-

national Centrifuge using 7.0 ml. cups and the high speed adaptor head. The supernatant fluid was withdrawn and tested. A second absorption, if necessary, was made by treating the first absorbed serums with equal portions of absorbing antigen suspensions.

Agglutinin-absorption procedure II. The technique of this modified procedure was essentially the same as that described above with the following modifications: 1) the leptospiral cultures were maintained in a modified Stuart's medium containing no glycerin; 2) the sedimented leptospires were resuspended in isotonic buffered saline containing 0.25% formalin; 3) to avoid further significant dilutions of the serums, subsequent absorptions were performed with centrifuged packed leptospires, obtained by completely removing the supernatant fluid after centrifugation of the same quantity of antigen suspension used in the initial absorption. Routinely two successive absorptions were performed. Absorptions were considered complete when no macroscopic agglutination was evident in the absorbing mixture and agglutinins were completely removed by the homologous antigen. If necessary additional absorptions were performed with packed cells.

RESULTS

The initial characterization of the leptospiral isolates was based upon the cross agglutination-lysis reactions of the strains, employed as antigens, against a screening battery of antigenically distinct type-leptospiral immune rabbit serums, selected on the basis of previous classification studies (Gochenour and Yager, 1952). Subsequently, depending on their cross reactions, some of the strains were tested against additional type-leptospiral immune serums to disclose further relationships.

Within the limitations of a cross agglutination-lysis study, the isolates fell into seven distinct antigenic groups, composed of from one to six members. The cross-reactivity of the multiple isolates comprising distinct groups were identical. The results of the cross agglutination-lysis reactions of the leptospiral isolates are shown in Table 2. Members of groups I, II, V, VI, and VII appear to be related to the *pyrogenes*, *icterohemorrhagiae*, *grippotyphosa*, *hebdomidis* and *autumnalis* serogroups respectively, Strain H-6 (group II) shows strong serologic affinities for diverse serotypes and strain RR-1 (group III) appears to be more closely related to *L. benjamin* and *L. schüffneri*.

Immune rabbit serums were prepared against representative members of each group of leptospiral isolates for further serologic investigations. In view of the fact that strains H-5 and RW-6 were isolated from different sections of Southeast Asia and that some members of the serogroup *icterohemorrhagiae* may not be differentiated by cross-agglutination studies (unpublished data), both strains were studied. Cross agglutinin-absorption studies on the isolates were conducted against those serotype leptospiral strains with which they exhibited strong agglutination-lysis activity. The cross agglutinin-absorption reactions of the serotype strains employed in the characterization of the leptospiral isolates were performed only in those instances where the antigenic inter-relationships of these strains had not been previously studied in our laboratory.

TABLE 2

Cross agglutination-lysis reactions of the leptospiral isolates

IMMUNE SERUMS	HOMOLOGOUS TITER	ANTIGEN = ISOLATES						
		I H-1,H-2 H-3,H-4	II H-6	III RR-1	IV H-5 RM-6	V RM-2	VI H-7,H-8 RM-3,RM-4 RS-5	VII BB-7,BB-8 BB-9,BB-10, BB-11,BB-12
a. *andaman*	25600	-	-	-	-	-	-	-
semaranga	25600	-	-	-	-	-	-	-
djatzi[c]	25600	-	-	-	-	-	-	-
hyos	25600	-	-	-	-	-	-	-
pomona	6400	-	-	-	-	-	-	400
djasiman	25600	-	-	-	-	-	-	-
sentot	25600	-	-	-	-	-	-	6400
autumnalis AB	25600	-	-	-	100	100	-	25600
autumnalis, Ft. Bragg	25600	-	-	-	-	400	-	25600
australis A, Ballico	6400	-	-	-	-	-	-	-
ballum	25600	-	-	-	-	-	-	-
canicola, Ruebush	25600	400	1600	1600	1600	1600	-	-
ictero. AB, Wijnberg	102400	100	100	100	102400	-	-	100
australis B, Zanoni	25600	6400	25600	-	100	-	-	-
alexi[d]	6400	6400	-	-	-	-	-	-
grippotyphosa, Mosc. V	25600	-	-	-	-	25600	-	-
medanensis	6400	-	-	-	—	-	6400	-
javanica	25600	-	-	-	-	-	-	-
b. *ictero.* A, Kant.	25600		6400	6400	25600			
naam	102400		6400	1600	102400			
maskarso	102400		6400	100	409600			
schuffneri	25600		1600	25600	1600			
benjamin	25600		102400	25600	400			
sarmin	25600		25600	-	6400			
pyrogenes, Salinem	25600	25600	400	-	-			
hebdomidis	25600						100	
wolffi	102400						102400	
sejroe	25600						1600	
saxkoebing	6400						1600	
hardjo	25600						6400	
borincano[e]	25600						1600	

a. Screening type-immune serums.
b. Additional type-immune serums.
c. Member of the serogroup *bataviae*.
d. Member of the serogroup *pyrogenes*.
e. Member of the serogroup *hebdomidis*.

TABLE 3

Cross agglutinin-absorption study on strain H-1

ANTISERUM		ANTIGEN			
Against	Absorbed with:	*pyrogenes*, Salinem	*australis* B, Zanoni	*alexi*	H - 1
pyrogenes, Salinem	Unabsorbed	6400	1600	6400	6400
	pyrogenes, Salinem	-	-	-	-
	australis B, Zanoni	6400	-	1600	
	alexi	1600	400	-	
	H - 1	-			-
australis B, Zanoni	Unabsorbed	6400	25600	6400	6400
	pyrogenes, Salinem	-	6400	-	-
	australis B, Zanoni	-	-	-	-
	alexi	100	6400	-	
	H - 1		6400		-
alexi	Unabsorbed	6400	6400	6400	6400
	pyrogenes, Salinem	-	-	6400	
	australis B, Zanoni	400	-	6400	
	alexi	-	-	-	-
	H - 1			6400	-
H - 1	Unabsorbed	25600	6400	6400	25600
	pyrogenes, Salinem	-			-
	australis B, Zanoni		-		25600
	alexi			-	6400
	H - 1	-	-	-	-

The results of agglutinin-absorption studies on strain H-1 with members of the *pyrogenes* serogroup (Table 3) indicates that this isolate is serologically identical with *L. pyrogenes*, Salinem. The results shown in Table 3 also verify the heterologous relationship between *L. australis* B, Zanoni and *L. pyrogenes*, Salinem (Wolff, 1952) and likewise reaffirm the distinct serologic relationships of the leptospiral isolate *L. alexi*, obtained from a human case in Puerto Rico (unpublished data), to the other members of the *pyrogenes* serogroup.

TABLE 4

Cross agglutinin-absorption study on strain RR - 1

ANTISERUM		ANTIGEN		
Against	Absorbed with:	RR - 1	*schuffneri*	*benjamin*
RR - 1	Unabsorbed	25600	6400	6400
	RR - 1	-	-	-
	schuffneri	-	-	-
	benjamin	1600	1600	-
schuffneri	Unabsorbed	6400	6400	1600
	RR - 1	-	-	-
	schuffneri	-	-	-
	benjamin	1600	400	-
benjamin	Unabsorbed	6400	6400	6400
	RR - 1	-	-	1600
	schuffneri	-	-	1600
	benjamin	-	-	-

TABLE 5

Cross agglutinin-absorption studies on strain H-6

ANTISERUM		ANTIGEN							
Against	Absorbed with	*ictero.*, Kant.	*naam*	*canicola*, Ruebusch	*austr.* B, Zanoni	*schuffneri*	*sarmin*	*benjamin*	H-6
ictero. A, Kantorowicz	Unabsorbed	25600							6400
	ictero., Kant.	-							400
	H-6	25600							-
naam	Unabsorbed		25600						1600
	naam		100						-
	H-6		102400						-
canicola, Ruebusch	Unabsorbed			25600					1600
	canicola, Rue.			-					-
	H-6			25600					-
australis B, Zanoni	Unabsorbed				25600				25600
	australis B, Zan.				-				-
	H-6				25600				-
schuffneri	Unabsorbed					6400			1600
	schuffneri					-			-
	H-6					6400			-
sarmin	Unabsorbed						1600		1600
	sarmin						-		-
	H-6						1600		-
benjamin	Unabsorbed							6400	6400
	benjamin							-	-
	H-6							6400	-
H-6	Unabsorbed	1600	1600	1600	400	400	1600	1600	25600
	ictero., Kant.	-							25600
	naam		-						25600
	canicola, Rue.			-					25600
	austr. B, Zan.				-				25600
	schuffneri					-			25600
	sarmin						1600*		25600
	benjamin							-	25600
	H-6	-	-	-	-	-	-	-	-

* Incompletely absorbed.

The results of the cross agglutinin-absorption studies shown in Table 4 reveal that RR-1 is serologically indistinguishable from *L. schüffneri*. It should also be noted that absorption with *L. benjamin* antigen effects a 16-fold reduction in the homologous titers of RR-1 and *L. schüffneri* antiserums, and suggests the incorporation of these serotypes into one serogroup.

Although strain H-6 demonstrated strong agglutination-lysis activity with a multiplicity of serotype strains, cross agglutinin-absorption studies (Table 5) indicated that the antibodies associated with serotype strain specificity were unaffected when absorbed with H-6 antigen and similarly absorption of H-6 agglutinins with the different serotype strain antigens resulted in no reduction in homologous titer. The serologic behavior exhibited by strain H-6 has not been described for any leptospiral isolate and consequently this strain constitutes a new leptospiral serotype.

The antigenic relationships of strains H-5 and RW-6 to the members of the *icterohemorrhagiae* serogroup are shown in Table 6. The homologous titer of the *L. mankarso* immune serum is completely removed when absorbed with strain H-5 and reduced by approximately 1000-fold when absorbed with RW-6. Absorption of the H-5 and RW-6 agglutinins by *L. mankarso* results in both instances in a 64-fold reduction in homologous titers. It is also noteworthy that cross absorption by *L. icterohemorrhagiae* AB, Wijnberg and RW-6 likewise re-

TABLE 6

Cross agglutinin-absorption studies on strains H-5 and RW-6

ANTISERUM		ANTIGENS					
Against	Absorbed with	*ictero.* AB, Wijnberg	*ictero.* A, Kant.	*naam*	*mankarso*	H-5	RW-6
ictero. AB, Wijnberg	Unabsorbed	102400				102400	102400
	ictero. AB,Wijn	-				-	-
	H-5	6400				-	
	RW-6	1600					-
ictero. A, Kantorowicz	Unabsorbed		25600			6400	25600
	ictero. A,Kant.		100			100	100
	H-5		25600			-	
	RW-6		25600				-
naam	Unabsorbed			102400		25600	102400
	naam			1600*		400*	1600*
	H-5			6400		-	
	RW-6			6400			100
mankarso	Unabsorbed				409600	409600	409600
	mankarso				-	-	-
	H-5				-	-	
	RW-6				400*		100*
H-5	Unabsorbed	6400	6400	25600	25600	25600	
	ictero. AB,Wijn	-				6400	
	*ictero.*A,Kant.		100			25600	
	naam			400		6400	
	mankarso				-	400	
	H-5	100	100	-	-	-	
RW-6	Unabsorbed	102400	6400	102400	102400		102400
	ictero. AB,Wijn	-					1600
	ictero. A,Kant.		-				409600
	naam			400			25600
	mankarso				-		1600
	RW-6	400	100	400	-		100

* Incompletely absorbed.

TABLE 7

Cross agglutinin-absorption studies on strain H-7

ANTISERUM		ANTIGEN						
Against	Absorbed with	*borincano*	*hebdomidis*	*sejroe*, Mallersdorf	*saxkoebing*	*medanensis*	*wolffi*	H-7
borincano	Unabsorbed	25600	6400	1600	6400	1600	400	1600
	borincano	-	-	-	-	-	-	-
	hebdomidis	25600	-	1600	1600	-	-	200
	sejroe, Mallersdorf	25600	6400	-	1600	200	400	400
	saxkoebing	25600	25600	6400	-	-	200	400
	medanensis	25600	6400	400	1600	-	400	400
	wolffi	25600	25600	400	1600	-	-	-
	H-7	25600	6400	400	1600	-	-	-
hebdomidis	Unabsorbed	6400	25600	1600	6400	1600	-	-
	borincano	-	6400	400	1600	400	-	-
	hebdomidis	-	-	-	-	-	-	-
	sejroe, Mallersdorf	6400	25600	-	1600	400	-	-
	saxkoebing	6400	6400	-	-	200	-	-
	medanensis	1600	25600	-	200	-	-	-
	wolffi	1600	25600	1600	6400	1600	-	-
	H-7	1600	25600	400	6400	1600	-	-
sejroe, Mallersdorf	Unabsorbed	-	1600	25600	1600	6400	1600	1600
	borincano	-	200	25600	1600	6400	400	1600
	hebdomidis	-	-	25600	1600	6400	400	1600
	sejroe, Mallersdorf	-	-	-	-	-	-	-
	saxkoebing	-	-	6400	-	1600	200	400
	medanensis	-	-	6400	1600	-	-	-
	wolffi	-	400	25600	1600	1600	-	-
	H-7	-	1600	25600	1600	6400	-	-
saxkoebing	Unabsorbed	400	1600	6400	102400	6400	400	400
	borincano	-	1600	6400	102400	6400	400	400
	hebdomidis	-	-	1600	102400	1600	400	400
	sejroe, Mallersdorf	200	400	-	102400	-	-	-
	saxkoebing	-	-	-	400	-	-	-
	medanensis	400	400	1600	25600	200	-	-
	wolffi	400	400	1600	102400	400	-	-
	H-7	400	1600	1600	102400	400	-	-

TABLE 7—*concluded*

Against	Absorbed with	*borincano*	*hebdomidis*	*sejroe*, Mallersdorf	*saxkoebing*	*medanensis*	*wolffi*	H-7
medanensis	Unabsorbed	1600	1600	25600	6400	102400	6400	6400
	borincano	-	1600	6400	1600	25600	6400	6400
	hebdomidis	-	-	6400	400	25600	6400	6400
	sejroe, Mallersdorf	-	400	-	-	6400	400	1600
	saxkoebing	200	1600	6400	-	25600	6400	6400
	medanensis	-	-	-	-	400	-	-
	wolffi	-	1600	6400	6400	25600	-	400
	H-7	-	400	6400	1600	25600	-	-
wolffi	Unabsorbed	400	400	6400	400	6400	25600	25600
	borincano	-	-	6400	400	25600	25600	25600
	hebdomidis	-	-	6400	400	25600	25600	25600
	sejroe, Mallersdorf	200	200	-	-	6400	25600	25600
	saxkoebing	200	200	1600	-	6400	25600	25600
	medanensis	-	200	200	-	200	6400	6400
	wolffi	-	-	-	-	-	-	-
	H-7	-	-	-	-	-	1600	-
H-7	Unabsorbed	400	400	400	-	400	6400	6400
	borincano	-	-	200	-	200	1600	6400
	hebdomidis	-	-	-	-	400	1600	6400
	sejroe, Mallersdorf	-	-	-	-	-	1600	6400
	saxkoebing	200	-	200	-	400	1600	6400
	medanensis	-	-	-	-	-	1600	6400
	wolffi	-	-	-	-	-	-	-
	H-7 H-7	-	-	-	-	-	-	-

sults in a 64-fold reduction in their respective homologous titers. A re-evaluation of the relationships of *L. mankarso*, *L. icterohemorrhagiae* and strain RW-6 within the framework of the classification system of Wolff and Broom (1954) is indicated.

In conducting a cross agglutinin-absorption analysis of strain H-7 with the members of the *hebdomidis* serogroup, we investigated at this same time the serologic interrelationships among the serotype members of the *hebdomidis*

TABLE 8

Additional cross agglutinin-absorption studies on strain H-7

ANTISERUM		ANTIGEN		
Against	Absorbed with:	*hardjo*	*wolffi*	H-7
hardjo	Unabsorbed	6400	1600	6400
	hardjo	-	-	-
	wolffi	1600	-	-
	H-7	1600	-	-
wolffi	Unabsorbed	25600	25600	25600
	hardjo	-	25600	6400
	wolffi	-	-	100
	H-7	-	6400	-
H-7	Unabsorbed	1600	6400	6400
	hardjo	-	1600	1600
	wolffi	-	-	-
	H-7	-	-	-

TABLE 9

*Cross agglutinin-absorption studies on strain RM-2**

ANTISERUM		ANTIGEN		
Against	Absorbed with:	*grippo.*, Mosc V	*bovis*	RM-2
grippotyphosa, Moscow V	Unabsorbed	6400	25600	25600
	grippo., Mosc V	-	-	-
	bovis	200	-	-
	RM-2	200	-	-
grippotyphosa, *bovis*	Unabsorbed	25600	25600	25600
	grippo., Mosc V	-	-	-
	bovis	-	-	-
	RM-2	200	200	-
RM-2	Unabsorbed	25600	25600	25600
	grippo., Mosc V	-	-	1600
	bovis	200	-	1600
	RM-2	-	-	-

* Agglutinin-absorption procedure I used.

TABLE 10

Cross agglutinin-absorption study on strain BB-7

ANTISERUM		ANTIGEN				
Against	Absorbed with	*autumnalis*, Ft. Bragg	*autumnalis*, Aki. A.	*autumnalis*, Rachmat	*bangkinang*	BB - 7
autumnalis, Ft. Bragg	Unabsorbed	25600				6400
	autumnalis, Ft. Bragg	-				-
	autumnalis, Aki. A.	1600				
	autumnalis, Rachmat	25600				
	bangkinang	6400				
	BB-7	1600				-
autumnalis, Akiyami A	Unabsorbed		25600			25600
	autumnalis, Ft. Bragg		400			
	autumnalis, Aki. A.		-			-
	autumnalis, Rachmat		6400			
	bangkinang		25600			
	BB-7		100			-
autumnalis, Rachmat	Unabsorbed			6400		6400
	autumnalis, Ft. Bragg			400		
	autumnalis, Aki. A.			400		
	autumnalis, Rachmat			-		-
	bangkinang			400		
	BB-7			1600		-
bangkinang	Unabsorbed				6400	6400
	autumnalis, Ft. Bragg				1600	
	autumnalis, Aki. A.				1600	
	autumnalis, Rachmat				6400	
	bangkinang				-	-
	BB-7				1600	-
BB-7	Unabsorbed	25600	6400	1600	1600	25600
	autumnalis, Ft. Bragg	100				100
	autumnalis, Aki. A.		400*			100*
	autumnalis, Rachmat			-		6400
	bangkinang				-	6400
	BB-7	-	-	-	-	-

* Incompletely absorbed.

group (with the exception of *L. hardjo*) and included the heterologous serotype member *L. borincano*, isolated from a human case of leptospirosis in Puerto Rico (unpublished data). The results of these studies shown in Table 7 confirm the classification of these strains as serologic heterologous serotypes (Wolff and Broom, 1954). The cross reactivity of the absorbed serums with each of the serotype strains has been included in the body of the data as additional information of the relationships among the strains. The Malayan isolate, H-7 is closely related to *L. wolffi*. Agglutinins prepared against strain H-7 are completely removed by absorption with *L. wolffi* antigen, while the absorption of *L. wolffi* antiserum with H-7 results in a 16-fold reduction in homologous titer. The titers of the *wolffi* and H-7 antigens against the other absorbed and unabsorbed serotype strains are remarkably parallel; however, the serologic disparity between the two strains is brought out by the differences in the reactivity of their absorbed antiserums against the *sejroe* and *medanensis* antigens.

Agglutinin-absorption procedure I was employed in the study shown in Table 7. Subsequently, with the acquisition of the *hebdomidis* serogroup member *L. hardjo*, additional absorption studies were conducted with this strain, isolate H-7, and *L. wolffi*. Although the modified agglutinin-absorption procedure was used, the results shown in Table 8 substantially verified the previously elucidated relationship between strain H-7 and *L. wolffi* and demonstrated that *L. hardjo* was antigenically heterologous with respect to the other two strains.

Table 9 gives the results of absorption studies with strain RM-2 and two strains of *L. grippotyphosa*. The serologic relationship between the two serotype strains is consistent with previous findings (Wolff and Bohlander, 1952). Absorption of strain RM-2 immune serum with either of the *L. grippotyphosa* strains reduces the homologous titer 16-fold. Absorption of both the Moscow V and Bovis antibodies with RM-2 leaves a small residual homologous titer.

The antigenic analysis of strain BB-7 with the serotype members of the *autumnalis* groups, including the Fort Bragg strain (Alexander *et al.*, 1954) is shown in Table 10. Except for very minor differences, strain BB-7 is serologically identical with *L. autumnalis* AB, Akiyami A. The antigenic relationships of the *autumnalis* serogroup members have been described in another report (Alexander *et al.*, 1954).

DISCUSSION

The cross agglutination-lysis patterns of the isolates could in all but two instances (H-6, RR-1) relate these strains to established leptospiral serogroups. In no instance could serotype identification be established with certainty. The results of our studies re-emphasize the importance of cross agglutinin-absorption procedures for definitive serologic typing of leptospiral strains.

In the serologic characterization of strains isolated from Southeast Asia, we have adopted the scheme of Wolff and Broom (1954) in the classification of leptospires with some modifications (Alexander *et al.*, 1954). With the techniques employed in our studies, two strains are considered heterologous if the antiserum of each strain following absorption by the other strain retains at least

1/16 of the homologous titer. Similarly two strains are homologous if the residual titers following absorptions are less than 1/16 of the original homologous titers. The designation of "complete" and "incomplete" biotypes are likewise determined by these standards. The application of these criteria to our agglutinin-absorption studies reveals that:

1. Strain H-1 is serologically homologous to *L. pyrogenes*, Salinem.
2. Strain RR-1 is serologically homologous to *L. schüffneri*.
3. Strain H-6 is a hitherto unrecognized new serotype, sharing major antigenic components with diverse leptospiral serotype strains.
4. Both strain H-5 and RW-6 are serologically homologous to *L. mankarso*.
5. Strain H-7 is an "incomplete" biotype of *L. wolffi*.
6. Strain RM-2 is the "complete" biotype of *L. grippotyphosa*.
7. Strain BB-7 is serologically homologous to *L. autumnalis* AB, Akiyami A.

The serologic characteristics of three of the isolates have not, to the authors knowledge, been previously described. We therefore propose designation of strain H-6 as *L. malaya*, strain H-7 as *L. wolffi* A, and strain RM-2 as *L. grippotyphosa* AB.

SUMMARY

A serologic study of twenty leptospiral isolates from Malaya, North Borneo and Thailand is reported. Initial serologic typing of these strains by means of the cross agglutination-lysis reactions revealed the presence of seven distinct antigenic leptospiral types. Subsequent antigenic analysis of representative strains indicated the presence in Malaya of *L. pyrogenes*, Salinem (4 human), *L. mankarso* (1 human), and an "incomplete" biotype of *L. wolffi* (2 human, 3 rodent), a "complete" biotype of *L. grippotyphosa* (1 rodent), *L. schüffneri* (1 rodent) and a new serotype strain (1 human). In addition *L. mankarso* was isolated from *R. whiteheadi* trapped in North Borneo, and *L. autumnalis* AB was isolated from the six members of the rodent genus *Bandicoota*, trapped in Thailand.

Classification of the leptospiral isolates based on a modified adoption of the proposed scheme of Wolff and Broom (1954) disclosed the presence of three hitherto undescribed antigenic types, a new serotype designated as *L. malaya*, an "incomplete" biotype of *L. wolffi*, designated as *L. wolffi* A, and a "complete" biotype of *L. grippotyphosa* designated as *L. grippotyphosa* AB.

The results of agglutinin absorption studies with the members of the *pyrogenes*, *icterohemorrhagiae*, *hebdomidis*, and *grippotyphosa* serogroups were consistent with the findings of other investigators on the inter-relationships among the serotype members of these respective groups.

REFERENCES

1. Alexander, A., Evans, L. B., Jeffries, H., Gleiser, C., and Yager, R. H., 1954. Serologic characterization of the Fort Bragg leptospire, *Proc. Soc. Exper. Biol. & Med.* **86**: 405–408.
2. Broom, J. C., 1953. Leptospirosis in tropical countries. A review, *Trans. Roy. Soc. Trop. Med. Hyg.* **47**: 273–291.

3. Collier, W. A., 1948. The Verbreitung der Leptospiren in Niederländische Indien, *Acta Trop., Basel* **5**: 135–159.
4. Fletcher, W., 1928. Recent work on leptospirosis, tsutsugamushi disease and tropical typhus in the Federated Malay States, *Trans. Roy. Soc. Trop. Med. Hyg.* **21**: 265–288.
5. Gochenour, W. S., Jr., and Yager, R. H., 1952. Classification of North American leptospires, *Proc. A.V.M.A.* 178–183.
6. Gochenour, W. S., Jr., Yager, R. H., Wetmore, P. W., and Hightower, J. H., 1953. Laboratory diagnosis of leptospirosis, *Am. J. Pub. Health* **43**: 405–410.
7. Lingen, F. F. L., 1933. Enkele gewallen van leptospirosis in Oelee-Soengai, *Geneesk. Tijdschr v. Ned. Indië* **73**: 1073–1077.
8. Schüffner, W., 1918. Ueber infectiosen Icterus und uber einen Spirenchätenbefund bei einer klinisch als Schwarzwasserfieber verlaufenden Erkrankung, *Geneesk. Tijdschr. v. Ned. Indië* **58**: 352–373.
9. Stuart, R. D., 1946. The preparation and use of a simple culture medium for leptospirae. *J. Path. and Bact.* **58**: 343–349.
10. Sundharagiati, B., and Buspavanich, S., 1951. A study on leptospirosis, *J. Med. Assoc. Thailand* **34**: 39–60.
11. Walch-Sorgdrager, B., 1939. Leptospirosis, *League of Nations Bull. Health Organization* **8**: 143–386.
12. Wisseman, C. L., Traub, R., Gochenour, W. S., Jr., Smadel, J. E., and Lancaster, W. E., 1955. Leptospires of man and animals in urban, rural and jungle areas of Southeast Asia, *Am. J. Trop. Med. and Hyg.* **4**: 29–40.
13. Wolff, J. W., and Broom, J. C., 1954. The genus *Leptospira* Noguchi, 1917. Problems of classification and a suggested system based on antigenic analysis., *Doc. de Med. Geograph. et Tropica* **6**: 78–95.
14. Wolff, J. W., 1952. The classification of pathogenic leptospires. Symposium on the leptospiroses, *Medical Sci. Publ.* No. **1**: 174–182.
15. Wolff, J. W., and Bohlander, H., 1952. Bovine leptospirosis, *Doc. de Med. Geograph. et Tropica* **4**: 257–265.

TREATMENT OF THE ACUTE FEBRILE PHASE OF CARRIÓN'S DISEASE WITH CHLORAMPHENICOL[1]

OSCAR URTEAGA B. AND EUGENE H. PAYNE

Departmento de Anatomia Patologica del Laboratorio Oswaldo Hercelles, Lima, Peru, and Department of Clinical Investigation, Parke, Davis and Company

Carrión's disease is endemic in the narrow valleys of the Andes Mountains within definite altitude limits. The disease is confined to Peru, with occasional short appearances in Ecuador, Chile, and Colombia (Pinkerton, 1951; DeVault, 1952). Sometimes the disease appears in places where it is very unlikely that the vector *Phlebotomus noguchi* or *P. verrucarum* could have fed on man; therefore a plant reservoir (*Jatropha macrantha, J. basiacantha*) was suspected. However, Herrer (1953) showed that these plants do not serve as reservoirs for the *Bartonella bacilliformis*.

A precipitous drop in red blood cell count is characteristic of the acute febrile phase of Carrión's disease. Red cell count may fall from normal to less than 1,000,000 in four or five days—a decline of a million red cells a day. With the exception of actual hemorrhage, this rate of loss of red cells is rarely equaled in other conditions.

Peripheral macrocytic anemia and a megaloblastic hyperplasia of the bone marrow develop. In the advanced stages of the disease the bartonellae move into the endothelial cells. Clusters of bartonellae may be found within swollen endothelial cells. Carrión's disease in the acute stage produces fever up to 104°F., muscle and joint pain, headache, and insomnia, with delirium and coma as terminal manifestations. Patients show progressive pallor, emaciation, prostration, rapid pulse, and dyspnea (Pinkerton, 1951). In the severe form of the disease mortality has been 90 to 95 per cent. Secondary bacterial infection, particularly with *Salmonella*, has been emphasized as an important factor in fatal cases. When *Salmonella* infection occurs as a complication in Carrión's disease, rational treatment requires medication against both bartonellosis and salmonellosis. Chloramphenicol is specific for the bartonella organism, and is effective against *Salmonella* infections, as well.

Krumdieck (1949) was the first to use chloramphenicol in treating Carrión's disease. He reported encouraging results in the eruptive form of the disease. In 1949 Payne and Urteaga (1951) treated 6 patients in the anemic febrile phase of Carrión's disease with chloramphenicol by the oral route. Initial intravenous doses of chloramphenicol were given to those patients who were comatose. In all patients there was a rapid defervescence of temperature to normal within 24 hours after administration of the drug. Concurrent with return of normal temperature, delirium and systemic toxemia subsided and the blood pattern im-

[1] The Chloromycetin used for this study was supplied by the Department of Clinical Investigation, Parke, Davis and Company.

TABLE 1

Results of treatment with chloramphenicol of 19 cases of Carrión's disease

Hist. No. Age, Sex	Days from onset % of r.b.c. parasitized	R.b.c. Bone marrow hyperplasia (pre-treatment)	Treatment	Temperature returned to normal	R.b.c. Bone marrow hyperplasia, 20-40 da. post-treatment	Relapse Retreatment Complications	Results
H. 35, 28 yrs., M	16 95%	2,740,000 ++++	Chloramphenicol: 9.0 Gm./5 days	24 hrs.	4,500,000 ++++	Five days post-treatment. Chloramphenicol 18 Gm./4 days	Recovery
H. 280, 17 yrs., M	10 50%	1,970,000 ++++	IV. Chloramphenicol: 15.2 Gm./7 days	24 hrs.	3,900,000 +++		Rapid recovery
H. 306, 39 yrs., M	30 70%	2,310,000 +++	Chloramphenicol: 15 Gm./5 days	24 hrs.	4,200,000 ++		Rapid recovery
H. 309, 14 yrs., M	3 40%	1,500,000 +++	Chloramphenicol: 15 Gm./5 days	24 hrs.	4,420,000 ++		Rapid recovery
H. 347, 24 yrs., M	9 80%	2,220,000 +++	Chloramphenicol: 20 Gm./5 days	24 hrs.	4,440,000 +	Three days post-treatment. Chloramphenicol 12 Gm./3 days	Recovery
H. 401, 22 yrs., M	8 80%	1,460,000 ++++	Neomycin: 7 Gm./7 days. No results. Chloramphenicol: 17.25 Gm./6 days	— 48 hrs.	1,500,000 ++++	Salmonellosis	Died
H. 426, 44 yrs., F	16 80%	2,070,000 ++++	Cortisone: 1.4 Gm./6 days. No results. Chloramphenicol: 21 Gm./7 days	— 48 hrs.	4,200,000 +++		Rapid recovery
H. 427, 30 yrs., M	20 70%	2,500,000 +++	Cortisone: 1.4 Gm./days. No results. Chloramphenicol: 18 Gm./6 days	— 24 hrs.	2,950,000 ++		Rapid recovery

H. 428, 21 yrs., M	16 60%	1,240,000 +++	Chloramphenicol: 15 Gm./5 days	24 hrs.	2,670,000 ++		Gradual recovery
H. 431, Adult, M	14 95%	2,200,000 +++	Chloramphenicol: 21 Gm./7 days	24 hrs.	4,000,000 +++		Rapid recovery
H. 433, 22 yrs., M	15 90%	1,900,000 ++	Cortisone: 1.2 Gm./4 days. No results.	—	3,800,000 +		Rapid recovery
			Chloramphenicol: 15.5 Gm./5 days	24 hrs.			
H. 434, 25 yrs., M	15 80%	0,850,000 ++++	I.V. & Oral Chloramphenicol: 14 Gm./5 days	24 hrs.	3,000,000 +++		Rapid recovery
H. 436, 28 yrs., M	15 80%	1,880,000 +++	Chloramphenicol: 18 Gm./6 days	Lysis	2,800,000 ++	Streptomycin 23 Gm. Salmonellosis	Slow recovery
H. 327, 36 yrs., M	11 90%	2,280,000 +++	Chloramphenicol: 18 Gm./6 days	48 hrs.	3,480,000 +++		Rapid recovery
H. 441, 18 yrs., M	15 80%	1,200,000 +++	Chloramphenicol: 9.5 Gm./3 days	48 hrs.	3,900,000 ++		Rapid recovery
			Streptomycin: 10 Gm./10 days	—			
H. 482, 20 yrs., M	7 40%	2,800,000 ++	Ilotycin: 30.4 Gm./12 days	—	4,820,000 +	Twenty days post-treatment. Chloramphenicol: 12 Gm./8 days Salmonellosis	Gradual recovery
			Aureomycin I.V.: 0.3 Gm./3 days	—			
			Chloramphenicol: 28.5 Gm./13 days	48 hrs.			
F. A., 18 yrs., M	8 90%	1,900,000 +++	Chloramphenicol: 15 Gm./5 days	24 hrs.	4,200,000 ++		Rapid recovery
H. 506, 20 yrs., M	14 100%	3,710,000 +++	Chloramphenicol: 15 Gm./5 days	24 hrs.	4,620,000 +++		Rapid recovery
P. Ch., 32 yrs., M	10 80%	2,200,000 +++	Chloramphenicol: 18 Gm./6 days	48 hrs.	3,900,000 ++		Rapid recovery

proved with marked reticulocytosis followed by a satisfactory rise in mature erythrocytes. None of the patients developed *Salmonella* infection.

This report, the second by these authors on chloramphenicol in Carrión's disease, concerns its use in an additional 19 patients in the anemic febrile stage of Carrión's disease. The patients ranged in age from 17 to 44 years, and one was a woman. Three patients had concurrent *Salmonella* infections at the time treatment was initiated. On admission, the patients were acutely ill. They showed pallor of skin and mucosa, rapid pulse, dyspnea, enlarged liver and spleen, and generalized lymph gland hypertrophy. Before treatment with chloramphenicol the patients had macrocytic anemia with a red blood cell count which varied from 850,000 to 3,710,000 with 40 to 100 per cent of the red cells parasitized. All patients showed a marked hyperplasia of the bone marrow.

TREATMENT AND RESULTS

An average total dose of 17.0 gm. of chloramphenicol in divided doses was administered orally to each of the 19 patients. Table 1 gives details on individual patient's age, sex, condition, treatment and response.

After the first administration of chloramphenicol, the patient's temperature, in most instances, returned to normal within 24 hours. In all patients reticulocyte response was prompt. Following chloramphenicol therapy, red blood cell counts and red cell size returned to normal. The bacillary form of the bartonellae disappeared from the red blood cells in 24 hours or underwent transformation to the coccoid form and then disappeared.

Of the 3 patients infected with *Salmonella*, one, moribund when admitted, died. This patient was first treated with neomycin during seven days without results. Forty-eight hours after chloramphenicol was started, the temperature became normal, but 24 hours later the fever returned and the patient died three days later. The other two patients made a gradual recovery. In the three relapses that occurred, additional dosages of chloramphenicol for three to eight days resulted in recovery. None of the patients showed evidence of intolerance to chloramphenicol therapy; no side reactions developed.

Frequent sternal punctures were made on all patients and also on numerous patients taking chloramphenicol as a prophylactic against Carrión's disease. Despite the relatively heavy dosage and the long-term prophylactic use, no deleterious influence was observed on the bone marrow. In the acutely ill patients, bone marrow was actively stimulated as evidenced by increased production of reticulocytes.

SUMMARY

Each of 19 patients undergoing the acute febrile phase of Carrión's disease received, in divided doses, approximately 17.0 gm. oral chloramphenicol in five days. After initial dosage with chloramphenicol, temperature usually returned to normal within 24 hours. A reticulocyte response averaging nine per cent (varying between 6.8% and 36%) was evident shortly after the beginning of chloramphenicol therapy. Although some coccoid forms of the bartonellae re-

mained in the red cells, the bacillary form disappeared in 24 hours. Red cell count and red cell size were reestablished. Relapse was infrequent; when it occurred, further administration of chloramphenicol resulted in recovery.

Three of the patients were also infected with *Salmonella*; one of these died, the other two made a gradual recovery. Since Carrión's disease and a *Salmonella* infection are likely to co-exist, chloramphenicol is suggested as the drug of choice to combat both the bartonellosis and the complicating salmonellosis. Large doses of chloramphenicol had no depressing influence on blood formation.

ADDENDUM

Since the above article was prepared Dr. Manuel Cuadra C. has published an excellent study entitled, "La complicación salmonelosica en la bartonelosis", *Revista Medica Peruana* **25**: 253 (Julio) 1954. From his careful study he concludes that the most suitable antibiotic available for the treatment of acute bartonellosis is chloramphenicol, which not only terminates the primary infection, but is effective against the grave danger of salmonellosis which is the cause of a high percentage of the deaths in Carrión's disease.

REFERENCES

DeVault, V. T., 1952. Treatment of bartonellosis with antibiotics, *Ann. N. Y. Acad. Sci.* **55**: 1222.

Herrer, A., 1953. Carrión's disease. I. Studies on plants claimed to be reservoirs of *Bartonella bacilliformis*, *Am. J. Trop. Med. Hyg.* **2**: 637.

Krumdieck, C. F., 1949. La enfermedad de Carión o verruga peruana en el niño, *Anales de la Fac. de Med. de Lima* **32**: 4.

Payne, E. H., and Urteaga, O., 1951. Carrión's disease treated with chloromycetin, *Antibiotics and Chemotherapy* **1**: 92.

Pinkerton, H., 1951. Carrión's disease, in Cecil and Loeb's *Textbood of Medicine*, Eighth Edition, W. B. Saunders Company, p. 106.

A SURVEY OF POLIOMYELITIS VIRUS ANTIBODIES IN FRENCH MOROCCO[1, 2]

JOHN R. PAUL AND DOROTHY M. HORSTMANN

The Section of Preventive Medicine, Yale University School of Medicine

The purpose of this survey has been to apply relatively new serological methods to obtain data on the prevalence of infection with poliomyelitis viruses in French Morocco. The Casablanca area was chosen because the recent influx of susceptible individuals coming annually into Casablanca from other countries has been and is appreciable.

One might raise the question as to whether or not information dealing with the prevalence of poliomyelitis could not have been obtained more satisfactorily, and much more easily, from official reports of the local Ministry of Health listing the annual numbers of cases. Experience in this matter has shown, however, that in some instances such reports are moderately accurate, whereas in others they are grossly inadequate, not necessarily because of under-reporting, but because poliomyelitis like a number of other diseases is less easy to recognize when it occurs in mild and sporadic forms. Thus, in the case of two common virus diseases, poliomyelitis (Paul *et al.*, 1944), and infectious hepatitis (Van Rooyen *et al.*, 1946), official reports from the Middle East and North Africa reflect a low incidence, quite out of keeping with the actual prevalence of these infections (Paul *et al.*, 1944, 1952) or the local prevalence of their respective viruses. In a way these "official reports" are correct, since they refer to the frank cases; but both of these diseases are endemic in the native populations in these areas, as opposed to epidemic, and occur commonly in infants and young children in whom they are apt to be milder and less easy to diagnose than in adults, although none the less infectious. It is not surprising therefore that when dealing with the common, sporadic, infantile and often inapparent forms of poliomyelitis or hepatitis, an enormous percentage of cases goes unnoticed, undiagnosed, and obviously unreported.

Epidemiologically, then, the situation is one in which there is a ready opportunity for the inadvertent spread of these "hidden" infectious diseases, transmitted largely by contact, from an immune or semi-immune native population to a less immune, immigrant population. Serological surveys can perhaps indicate to a better degree the extent to which some of these hidden infections, as well as the overt infections, exist.

Locale and population. The geographical area of French Morocco, in question here, has been limited to the city of Casablanca and its immediate surroundings. This city is one of the few coastal ports of French Morocco, situated on the

[1] Representing work carried out under the auspices of the Virus and Rickettsial Disease Commission, Armed Forces Epidemiological Board, Washington, D. C., and the Preventive Medicine Division, Dept. of the U. S. Air Force.

[2] Aided by a grant from the National Foundation for Infantile Paralysis.

northwest corner of the African continent. Politically it is at present administered by France as a protectorate. Climatically the coastal region is essentially tropical and "Mediterranean" in type, with hot, semi-arid summers, and seasonal variations in day time temperatures which range from an average of 55°F. in January to 85–90°F. in August. Both seasonal and daily fluctuations increase sharply as one goes eastward into the interior. The rainfall along the coastal area ranges between 14 and 21 inches, and this again lessens as one goes eastward, with maximum precipitation occurring between January and April, and little rain falling from May to September.

As to *population* data and *socioeconomic* conditions of the urban area of Casablanca, the population in 1947 was about 560,000; whereas in 1953, the year of the survey, it was approximately 655,000. Of these, 125,000 are Europeans, and 530,000 native Moroccans (450,000 "Mussulman" and 80,000 "Israelites"). The local Moroccans, who are both urban and village dwellers, have been classified as being of three ethnic types: Arab, Berber or Chleuh.

Living conditions vary greatly, the modern European sections being distinct from the native quarters or *medinas*. The native quarters are represented by a few new housing units but mainly by old crowded urban areas, or villages composed of huts. Most of these areas are in squalid surroundings. The traditional rural dwellings which can be found within a mile or two of the city vary from scattered primitive huts made of boughs, clay, or mud and wattle walls with thatched roof, to small groups or villages consisting of these structures enclosed in the form of a compound, surrounded by mud walls (Fig. 1).

In so far as possible, the blood samples were taken from healthy, native born members of the community (see Fig. 2). How representative our sample of 360 members who furnished the blood samples was of the population of Morocco as a whole, is something of a question. At least it can be said that the sample group consisted almost entirely of native born Moroccans of Arab stock (and a few Israelites), living in or near the city of Casablanca. Some of them were village residents; nearly all of them lived under conditions which would appear poor, primitive and often crowded to us, but normal to most of the local inhabitants.

Morbidity data. Infant mortality rates, generally regarded as a useful index of Public Health standards, are not available for French Morocco (Simmons *et al.*, 1953). It has been surmised, however, that they must be high in view of the primitive sanitary standards.

Poliomyelitis rates. During the past 20 years in French Morocco, poliomyelitis has been regarded as a sporadic and uncommon disease, the number of cases averaging about 13 per year. However, within the last few years (i.e. since 1950) the reported cases have increased to 50–60 per year, and it has been recognized recently that Americans and Europeans seem to be more susceptible than native born citizens. Since 1947 the recorded incidence in Casablanca has been that listed in Table 1.

The age distribution of the 39 cases occurring in Casablanca between Jan. 1 and Aug. 1, 1953 shown in Table 2 indicates marked differences between the

FIG. 1. Types of living conditions in and near the city of Casablanca (Photos taken in March 1953). Left: Street scene with dwellings in one of the poorer quarters of the city. Right: A compound within a very few miles of Casablanca showing straw huts and the children who inhabit them.

TABLE 1

*Poliomyelitis cases in Casablanca 1947–1953**

Year	European cases	Moroccan cases	Total cases
1947	2	0	2
1948	5	0	5
1949	4	0	4
1950	31	7	38
1951	29	4	33
1952	18	3	21
1953 (to 1 Aug.)	28	11	39

Moroccan and European groups with a limitation of the disease to young infants in the former group.

Mumps rates. Mumps has been chosen as a control disease for this survey because it is a virus disease usually regarded as being spread by human association. The age group distribution both of mumps cases and antibodies should therefore reflect the degree of crowding (or isolation) to which this population has been exposed. Clinical mumps is said to have the usual childhood incidence in French Morocco so far as prevalence and age groups attacked are concerned.

METHODS

Collection of blood specimens.[3] *Adult samples collected by one of us (J.R.P.) in March, 1953.*

With the permission of French Public Health authorities, blood samples were obtained in March, 1953, from 130 relatively healthy adult native born Moroccans from the neighborhood of Casablanca; 121 were males and 9 females. These samples were obtained from two sources: 1) Native laborers, applying for work at local U. S. Air Force installations, where these individuals were given physical examinations and at the same time it was required that blood be drawn for a VDRL test; and other native laborers who were reporting at the U. S. Air Force Industrial Clinic to be inoculated against typhoid and small

[3] We are primarily indebted to Dr. G. Sicault, Director of Public Health in French Morocco, and to the following French Public Health and Medical authorities: Dr. A. Messerlin, Bureau de la Santé Publique et de la Famille, Rabat; Dr. M. A. Merlin-Lemas, Medicine chef, Region de Casablanca; Dr. Jean Ritter, Medicine chef, Maurice Gaud Hospital, Casablanca; to Dr. Guidon, Medicine chef, Jules Mauran Hospital, Casablanca; and to Dr. J. Rutkowski, Medicine chef, Hygiene Service, Casablanca.

Thanks are also due to Dr. Georges Blanc, Director, and Dr. L. A. Martin, member of the Institut Pasteur, Casablanca.

We are also particularly grateful to Lt. Col. Jules B. Chapman, M.C., U.S.A.F., Commanding Officer, 7280th Medical Group Squadron, and to members of his staff, as well as to Col. H. G. Wallace, M.C., Air Surgeon. Particular thanks are due to Major H. A. McDonald, M.C. of the 7280th Epidemiological Flight, who was responsible for many of the arrangements necessary for carrying out the work. In addition, invaluable assistance was supplied by T/Sgt. Robert C. Pickett, Airman Wiley Rollins, and Dr. Pierre Roualt of Casablanca, in collecting blood specimens and separating sera.

TABLE 2

*Age distribution of Moroccan and European cases of poliomyelitis in Casablanca**

Age	Moroccan	European
0– 5 mos.	0	2
6–11 mos.	3	2
1 year	6	4
2 years	1	6
3 years	0	3
4 years	0	1
5– 9 years	1	5
10–14 years	0	1
15–19 years	0	0
20–29 years	0	1
30–39 years	0	3
Total	11	28

* These incidence figures have been obtained from the Hygiene Service, Casablanca, through its Medicine chef, Dr. J. Rutkowski.

pox. 2) Native born citizens of Casablanca and its environs who were undergoing delousing treatments at the Service d'Hygiene of Casablanca.

The age of each donor was not accurately stated, for Moroccans often do not keep track of their years. Consequently the ages of the adults are approximate, but the situation with young children is a little more accurate since the birth date could usually be calculated in relation to some feast such as Rhamadan or the Feast of the Mutton.

Juvenile samples, collected by one of us (D.M.H.) in Aug.–Sept. 1953.

These samples were also obtained by permission and with the help of French Health authorities. Most of the children from the Casablanca area who were bled belonged to the lowest socioeconomic group, and lived under extremely primitive and crowded conditions. The level of hygiene and cleanliness was poor; the babies often appeared dirty, thin, pale, and ricketty. The majority of them lived in the *Ancienne Medina*, which is the walled-in Mussulman quarter in the heart of the city, consisting of winding alley-width streets thronged with people night and day. Local sanitary facilities here were of the most primitive type: fecal wastes were collected several times weekly; water was obtained from various pumps scattered throughout the area. Flies were abundant.

The majority of blood specimens from infants were obtained at several social service or well-baby clinics, the Dispensaire Carrières Centrales, and Ben M'Sick. At these centers mothers receive instructions on infant care and feeding, babies are immunized, and minor medical and surgical problems are handled. Only children up to two years of age are admitted here. In general, 20 ml. of blood was taken from either the external jugular or femoral vein.

For the age group 2 to 6 years, the population of a nursery school for Mussul-

FIG. 2. Types of Moroccans, juvenile and adult, from whom blood samples were collected in Casablanca. (Photo by Dr. G. Blanc, Institut Pasteur, Casablanca who has graciously permitted its reproduction here.)

man children, which was operated in half of the building of the Dispensaire Carrières Centrales was used.

A number of specimens were also collected at the *Institut d'Hygiene*, from boys 6 to 18 years of age. These were all shoe-shine boys from Casablanca, who work in sidewalk cafes and roam all over the city. The boys are required to appear once weekly at the Institut d'Hygiene for a bath and delousing. Most of them live in the Ancienne Medina, although some were unable to give any address, stating merely that they slept behind such and such a building.

Additional specimens were obtained from patients in the Maurice Gaud and Jules Mauran hospitals of Casablanca, chiefly from traumatic cases, and a few from patients convalescent from minor infections. Placental bloods were obtained through the courtesy of the obstetrical staff of the Maurice Gaud hospital.

In all 230 juvenile specimens of blood were collected which, added to the adult group, brought the total collection to 360 blood samples. The age groups represented and the numbers in each, are indicated in Table 3.

Poliomyelitis antibody tests. Neutralizing antibodies to all three types of poliomyelitis virus were determined by tissue culture tests employing serum diluted

TABLE 3

Age distribution of 360 native-born adult Moroccans from whom blood samples were obtained

Juveniles		Adults	
Age	Number	Age	Number
0– 3 months	13	15–19 years	3
4– 6 months	11	20–24 years	42
7–11 months	19	25–29 years	46
1 year	36	30–34 years	21
2– 4 years	36	35–39 years	11
5– 9 years	41	40 years	7
10–14 years	42		
15–19 years	32		
Total	230		130
Grand total			360

1:5 (final 1:10) and 100 tissue culture doses of Type 1 (Brunhilde), Type 2 (Y-SK), and Type 3 (Leon) virus. These were carried out in monkey kidney epithelial outgrowth tubes.[4] The technique employed has been described (Ledinko *et al.*, 1952; Morann and Melnick, 1954).

Complement fixing antibodies against all three types of poliomyelitis virus were tested by the method of Black and Melnick (1954), using undiluted tissue culture antigens to the three types of virus, and serum diluted 1:5 and 1:15.[5] Much remains to be learned about the developments and interpretation of this test but it has reached a stage in which results can be satisfactorily used for the purposes of this study.

Serological tests used in mumps. Complement fixing antibodies were determined; a positive complement fixation test in a dilution of 1:4 has been interpreted as indicative of past experience with mumps virus. The technique used has been described (Army, 1949).[6]

RESULTS

Poliomyelitis antibody patterns. Data from both neutralizing and complement fixation tests appear in Table 4 and Figure 3. *Neutralizing* antibodies to all three types of poliomyelitis virus were present at birth in almost all of the 9 babies tested, the only exception being one infant who had Types 2 and 3 antibodies but not Type 1. At the age of 4–6 months these passively transferred maternal antibodies declined to 36%, 55% and 55% positive for Types 1, 2 and 3 respectively, with a greater decline for Types 2 and 3 by 7–11 months of age, where

[4] We are grateful to Sarah J. Melnick for performing these tests.

[5] We are grateful to Dr. J. L. Melnick and F. L. Black for running these complement fixation tests with Types 1, 2 and 3 poliomyelitis viruses.

[6] We are grateful to Dr. S. J. Liao for carrying out these complement fixation tests with mumps virus.

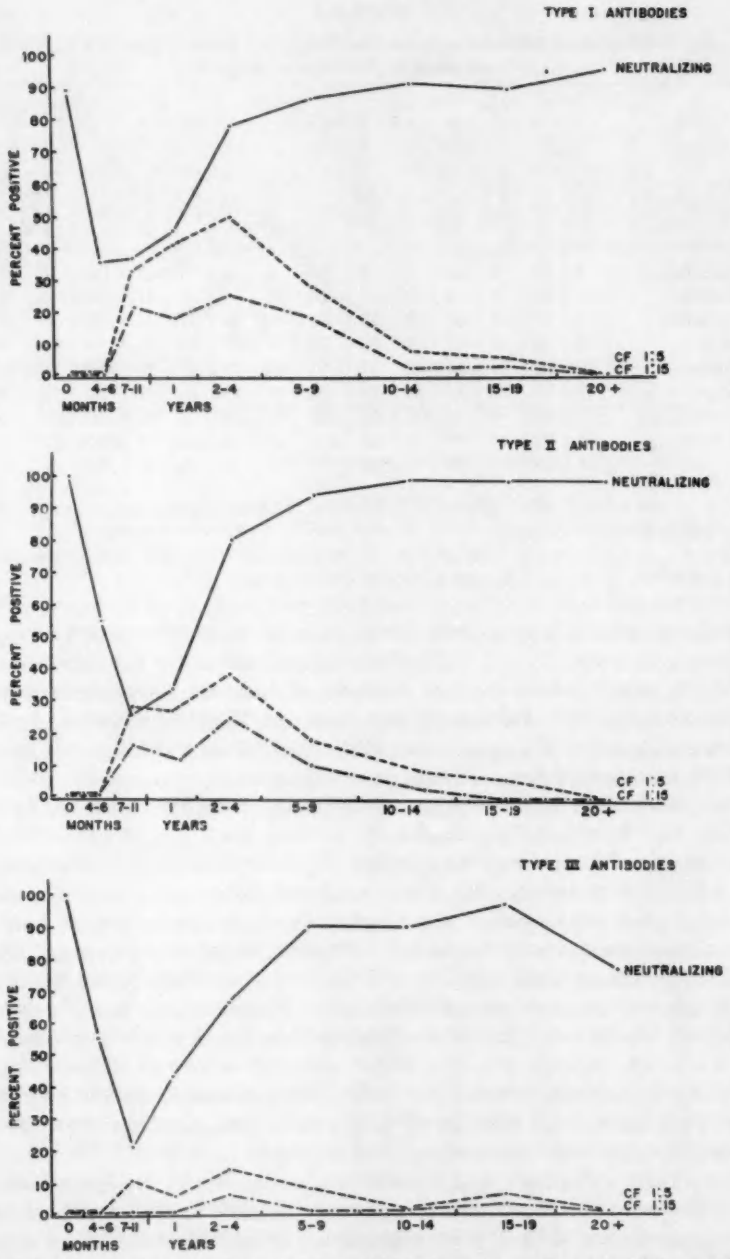


FIG. 3. Neutralizing and CF antibody patterns (Type 1 top, Type 2 middle, and Type 3 lower panel) according to various age groups, covering the period of 0 to 20+ years.

TABLE 4

Age distributional antibody patterns to poliomyelitis virus (Types I, II, & III) in blood samples from native moroccans

Age	Type I					Type II					Type III				
	Neutralizing*		CF†			Neutralizing*		CF†			Neutralizing*		CF†		
	Number tested	%+	Number tested	%+ 1:5	%+ 1:15	Number tested	%+	Number tested	%+ 1:5	%+ 1:15	Number tested	%+	Number tested	%+ 1:5	%+ 1:15
0 (placental)	9	89	9	0	0	9	100	9	0	0	9	100	9	0	0
4–6 months	11	36	11	0	0	11	55	11	0	0	11	55	9	0	0
7–11 months	19	37	18	33	22	19	26	18	28	17	19	21	18	11	0
1 year	35	46	34	41	18	35	34	34	27	12	35	43	34	6	0
2–4 years	36	78	36	50	25	36	81	36	39	25	36	66	36	14	6
5–9 years	41	87	39	28	18	41	95	38	18	11	41	90	38	8	0
10–14 years	41	92	40	8	3	41	100	40	8	3	41	90	40	0	0
15–19 years	32	90	32	6	3	32	100	32	6	0	32	97	32	6	3
20+	45	96	17	0	0	45	100	17	0	0	45	76	17	0	0

* %+ = per cent of sera (diluted 1:5) positive for neutralizing antibodies to types 1, 2, and 3 poliomyelitis viruses.

† %+ 1:5 and 1:15 = per cent of sera (diluted 1:5 and 1:15) positive for complement fixing antibodies to types 1, 2, and 3 poliomyelitis viruses.

the positives were 37, 26, and 21 per cents respectively (Table 4 and Fig. 3). The degree to which Type 1 antibody is maintained above the others and the speed with which it rises suggests that some infants are becoming infected almost immediately after the loss of their maternal Type 1 antibodies. Actually, however, a glance at Fig. 4 indicates that, if one considers the per cent of individuals in any age group having neutralizing antibodies to any one of the three types of virus, the level never falls below 58 per cent positive, which is found in the 7–11 month age group.

On the other hand, *complement fixing* (CF) antibodies to any one Type rose from 0 in the first few months of life to 33 per cent positive at 7–11 months, suggesting again that some of the infants, who had already lost or were just about to lose their passive maternal antibodies, had become infected almost immediately with at least one type of virus (but more often Type 1 or Type 2) and had thereby acquired an active immunity, evidenced by the rapid development of CF antibodies. The results illustrated in Fig. 3 point to the fact that there was ample poliomyelitis virus about, although as long as all these children retained their maternal neutralizing antibodies (i.e., during the first 6 months of life) the chances were that they would not become infected; but as soon as passive immunity was lost, active infection began.

Subsequently, between 1 and 4 years of age, it is apparent that a large per cent of the infantile population continued to acquire infection with all three types of virus, since in the 2–4 year age period 78%, 81%, and 66% of children possessed neutralizing antibodies to Type 1, 2, and 3, respectively (Table 4 and Fig. 3). When the neutralizing antibody curves are grouped comparatively

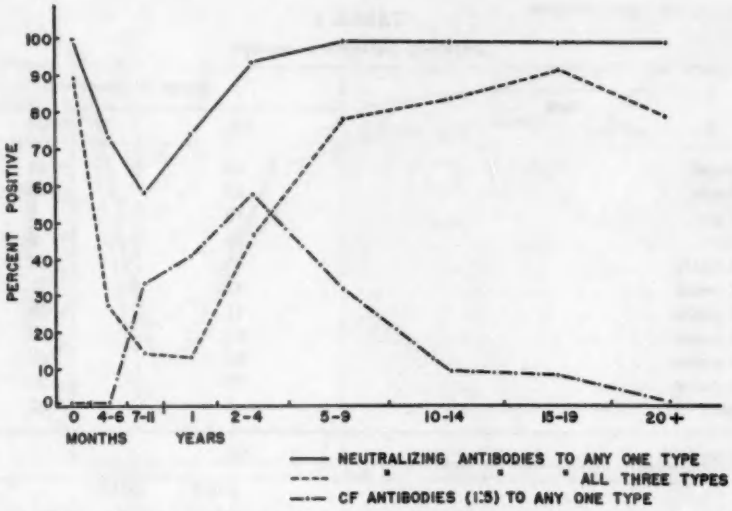


FIG. 4. Composite curves of neutralizing and CF antibodies to poliomyelitis in French Morocco.

(see Fig. 4 and 5) it appears that they rise together so that almost half the children of 2 to 4 years of age already have neutralizing antibodies to all three types, and by 5–9 years this reaches 78%.

As to the relationship between neutralizing and complement fixing antibodies in different age groups, this is also charted in Fig. 3 and 4. The curves are similar to those in a population in Northeast Africa previously studied for Type 2 only, (and by a different complement fixation method) by Goldblum and Melnick (1952). Thus while neutralizing antibodies, after an original fall, begin rising after six months of age, reach close to 100 per cent by the age of 5 and remain at that level in all older age groups, the presence of complement-fixing antibodies in this population is a childhood phenomenon, being absent during the first months of life and reaching a peak at 2–4 years, falling in the 5–9 year group, and existing only at low levels in the 10–14 year and older age groups. Since the complement fixing antibody in poliomyelitis is a transient one lasting probably on the average, not more than 3 years after infection, the curves suggest that active infection in the Moroccan population is taking place almost entirely in young children.

Mumps complement-fixation test (*see Table 5*). This antibody is also acquired at a fairly early age in the Moroccan population, in that by the time the children are 4 years old 50 per cent or more have been infected with mumps virus. The picture is comparable to that seen in Cairo, Egypt, and reflects a population in which a "crowd disease" spreads readily. It is again in some contrast to the situation in the United States or the European cities which have been similarly studied by Paul and Kraft (unpublished). In the latter populations children reach about 10 years of age before 50 per cent of them acquire mumps antibody.

TABLE 5

Antibody patterns to mumps

Ages	Mumps CF(1:4=+)	
	No.	%+
Placental	10	40
3– 6 mo.	11	10
7–11 mo.	19	5
1 year	35	9
2– 4 years	35	54
5– 9 years	42	71
10–14 years	41	59
15–19 years	34	62
20–29 years	74	55
30–39 years	27	70
40 years +	6	67
Total	334	

In other words, in the Moroccan population there is a high degree of exposure to mumps virus early in life which is presumably due to crowding. This close association of all age groups "pushes" this disease into earlier age groups than would be the case in an urban population in the United States. However, within the limits of the two methods, the age at which mumps is acquired in Morocco is not as early as the age at which poliomyelitis infection is acquired.

Comment. The findings indicate that, comparable to the population in Cairo, Egypt (Paul *et al.*, 1952), natural infection with poliomyelitis viruses and the development of antibodies to all three types comes early in life to children brought up in this urban or village environment of French Morocco. More than a third of the poliomyelitis infections with Types 1 and 2 come almost immediately after the loss of maternal antibody. This implies that all three types of poliomyelitis virus are heavily distributed throughout the local community and heavy exposure at an early age is the rule. How different this is from findings in the United States, where in nearly all areas studied to date (Paul *et al.*, 1952b), the acquisition of Type 2 neutralizing antibody (which is fairly representative of the other types) comes considerably later. The extent to which heavy infection in early childhood (without a high incidence of the recognized paralytic disease) creates a more solid type of immunity than that created by casual or more intermittent types of exposure and infection, such as one might find in a U. S. city situated at a comparable latitude, is a point on which further data are desirable. At least one can say that the age distributional antibody patterns for poliomyelitis and mumps, as well as the age at which these diseases are acquired, differ significantly in the Moroccan population and the U. S. cities studied.

As to the pertinence of the poliomyelitis complement fixation results in this connection, reference has already been made to the fact that much remains to be learned about this test. Nevertheless, the results can be accepted as indicating

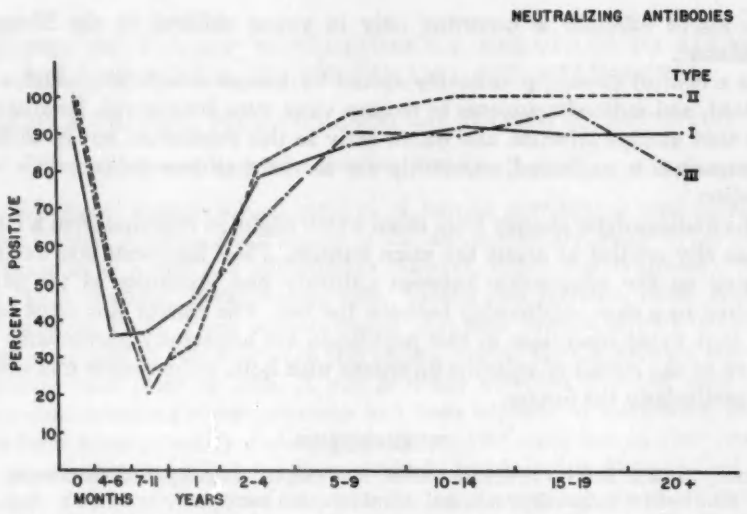


FIG. 5. Neutralizing antibodies to the three types of poliomyelitis virus in French Morocco.

that active poliomyelitis infection in the Moroccan population is taking place almost entirely in young children. This implies that by the age of 10 to 14 the juvenile population is through with the business of being infected with the three types of poliomyelitis virus. Adult infection or reinfection apparently seldom occur. The few young adults who have complement fixing antibodies may well represent that small group which happened to escape infection with a given type in childhood. Thus there is no reason to suppose that homotypic reinfection or so-called booster infections continue to occur in this population after school age is passed. Here again we have a situation quite different from that in the United States where adult infection is so common.

SUMMARY

A serological survey of antibodies to all three types of poliomyelitis virus has been carried out in an urban and semiurban population in French Morocco. The rates at which neutralizing antibodies and complement-fixing antibodies have been acquired by this population according to different age groups are herein reported.

Heavy infection and a rapid development of antibodies at an early age characterize the pattern obtained. The results indicate the presence of actively acquired neutralizing antibodies, and presumably immunity, to all three types of poliomyelitis virus in about 80 per cent of the population by the time children have reached 5 years of age. The percentages persist in all older age groups. Complement-fixing antibodies, on the other hand, are present largely in the juvenile population, and fall to low levels in those over 10 years of age. Since the complement fixing antibody in poliomyelitis is a transient one, this suggests

that active infection is occurring only in young children in the Moroccan population.

As a control disease, presumably spread by human association, mumps was selected, and antibody patterns to mumps virus were determined. Results indicate that mumps infection also comes early to this population but in so far as a comparison is warranted, apparently not as early as does poliomyelitis virus infection.

The findings differ sharply from those which might be expected from a United States city studied at about the same latitude. Their interpretation has some bearing on the relationship between antibody and immunity in the infant, pointing to a close relationship between the two. The results also point to the fact that living conditions in this population are apparently particularly conducive to the spread of infantile infections with both poliomyelitis and mumps but particularly the former.

REFERENCES

1. Army Medical Service Graduate School, 1949 (Sept.). Technique of complement fixation test for diagnosis of mumps. (Mimeographed pamphlet).
2. Black, F. L., and Melnick, J. L., 1954. The specificity of the complement fixation test in poliomyelitis, *Yale J. Biol. & Med.* **26**: 385–393.
3. Goldblum, N., and Melnick, J. L., 1952. Complement-fixing antibodies to Type 2 (Lansing) poliomyelitis virus in a normal population of a subtropical area, *J. Exp. Med.* **96**: 175–185.
4. Ledinko, N., Riordan, J. T., and Melnick, J. L., 1952. Multiplication of poliomyelitis viruses in tissue cultures of monkey testes. 1. Growth curves of type 1 (Brunhilde) and type 2 (Lansing) strains and description of a quantitative neutralization test, *Amer. J. Hyg.* **55**: 323–338.
5. Morann, G. L., and Melnick, J. L., 1953. Poliomyelitis virus in tissue culture. VI. Use of kidney epithelium grown on glass, *Proc. Soc. Exp. Biol. & Med.* **84**: 558–563.
6. Paul, J. R., Havens, W. P., and Van Rooyen, C. E., 1944. Poliomyelitis in British and American troops in the Middle East. The isolation of virus from human faeces, *Brit. M. J.* **1**: 841–843.
7. Paul, J. R., and Kraft, L. M., 1952. Complement-fixing antibody patterns to mumps virus in different populations. (Unpublished data).
8. Paul, J. R., Melnick, J. L., Barnett, V. H., and Goldblum, N., 1952. A survey of neutralizing antibodies to poliomyelitis virus in Cairo, Egypt, *Amer. J. Hyg.* **55**: 402–413.
9. Paul, J. R., Melnick, J. L., and Riordan, J. T., 1952. Comparative neutralizing antibody patterns to Lansing (Type 2) poliomyelitis virus in different populations, *Am. J. Hygiene* **56**: 232–251.
10. Simmons, J. S., Whayne, T. F., Anderson, G. W., Horack, H. M., and Thomas, R., 1951. *Global Epidemiology*. A Geography of Disease and Sanitation, Phila. J. B. Lippincott Co. **2**: 62–74.
11. van Rooyen, C. E., and Kirk, G. R., 1946. The spread of infective hepatitis and poliomyelitis in Egypt, *Edinburgh Med. J.* **53**: 529–543.

A SURVEY OF FOREST MOSQUITOES IN RELATION TO SYLVAN YELLOW FEVER IN THE PANAMA ISTHMIAN AREA[1]

HAROLD TRAPIDO, PEDRO GALINDO, AND STANLEY J. CARPENTER

Gorgas Memorial Laboratory, Panama, R. de P.

The results of immunological surveys of human populations conducted in Mexico, Central America and Panama in the late thirties and early forties have been reported in a series of publications (Sawyer, Bauer and Whitman, 1937; Clark, 1938; Sawyer, 1942; Bustamante, Kumm and Herrera, 1942; Kumm and Crawford, 1943). These authors record that such persons as were found to possess immunity to yellow fever in this region, west of the Canal Zone, were of age groups born prior to 1925. It was generally accepted that the older age groups demonstrating some immunity had been exposed to the disease in its urban form transmitted by *Aedes aegypti*. From 1905 until late in 1948 locally contracted yellow fever had not been clinically recognized in Panama, but the existence of naturally acquired immunity in the younger age groups at several places in Panama east of the Canal Zone was reported by Clark, 1938; Sawyer, 1942; and Kumm and Crawford, 1943. The interpretation of these data was that the sylvan phase of the disease (jungle yellow fever) had been present, and had gone unrecognized in the portion of Panama adjacent to South America and east of the Canal Zone, but that yellow fever was not endemic in Middle America west and north of central Panama and the Canal Zone. The present understanding of yellow fever as primarily a disease of tree-inhabiting anthropoids and possibly certain other mammals, with man only incidentally involved, indicated the desirability of an adequate survey of the forest canopy mosquito fauna in the critical Isthmian area to establish what elements determined the suitability of the forests of eastern Panama and the presumed unsuitability of the forests of western Panama for the propagation of sylvan yellow fever.

The need for such a study was emphasized by the occurrence of several fatal cases of sylvan yellow fever contracted just east of the Canal Zone late in 1948 (Herrera, Elton, and Nicosia, 1949). The writers at first undertook a study of the composition of the forest mosquito fauna in central Panama and the Canal Zone in the area where the new human cases had been recognized (Galindo, Trapido and Carpenter, 1950). Here we found two of the recognized vectors of the disease in South America, *Haemagogus spegazzinii falco* and *Aedes leucocelaenus*, present in some abundance. We also reported on the general distribution, the annual cycle of abundance, and the vertical stratification in the forest of these as well as all other mosquitoes attacking man in the forest in this area. Since this survey had shown the South American vectors to be present, the next year we extended these studies to western Panama and adjacent Costa Rica to establish whether or not there was a zoogeographic shift in the composition of the mosquito fauna of the forest canopy in the Isthmian area, marked by the end of

[1] Studies on the Forest Mosquitoes of Panama, VII.

the range of the South American vectors of the disease, which might be correlated with the supposed absence of the sylvan form of yellow fever from western Panama and Central America. We found at once that the change in the composition of the forest canopy mosquito fauna which, on a theoretical basis we had expected to demonstrate, was in fact lacking. We briefly reported the presence of *Haemagogus spegazzinii falco* in Puntarenas Province, Costa Rica, our westernmost collection station (Galindo, Carpenter and Trapido, 1951 a) but the full account of the findings of this study have been delayed until now by pressure of further field work on this general problem and by other activities. Subsequent to this study, sylvan yellow fever did in fact appear in western Panama and Costa Rica (Elton, 1952), and more recently in Nicaragua and Honduras. Observations on the forest mosquito faunas of these Central American countries will be the subject of later papers.

In Panama there is a considerable gradient in rainfall between the Caribbean and the Pacific drainages, both in terms of the total precipitation, and its distribution through the year. The Pacific slope has less rainfall, and a relatively severe dry season from January through April. On the Atlantic side of the Isthmus the dry season is less marked, and in some places there may be no month with less than four inches of rain. In consequence the Caribbean coast of Panama is largely a trackless tropical rain forest, uninhabited except by small groups of Indians, and accessible only by small coastal boats and by dugout cayucos up the larger rivers. West of the Canal Zone, the Pacific slope with its lesser rainfall and marked dry season is characterized by a lower and more scrubby forest ("deciduous tropical forest" of the plant ecologists) mixed with open grassy savannahs. Here the principal rural population of Panama lives, and there is a road passable at all times of the year from the Canal Zone almost to the Costa Rican border. While it would have been desirable to establish our series of stations for the capture of canopy mosquitoes in the true rain forest of the Caribbean slope, the impossibility of arranging for supply and the transportation of the collections into the laboratory on a routine basis made it necessary to operate for the most part in the best forest which could be found on the Pacific side. Apart from the series of stations located at intervals between the Canal Zone and Puntarenas Province in Costa Rica, we simultaneously set up several stations to the east, between the Canal Zone and Darien Province, adjacent to Colombia, for comparative purposes. We had learned from our previous experience in operating tree stations for a full year in central Panama and the Canal Zone, that most species of canopy mosquitoes, which are dependent for their larval development on accumulations of water in tree holes or epiphytic plants, were rare during the dry season months from February through April. As the present study was intended primarily as a survey, an economy of expenditures was effected by planning to carry on the collections only during the rainy season months between June and December. Certain of the stations, either because they were located in places difficult of access or because they failed to produce a significant catch, were run for shorter periods. At two places where secondary roads gave access to some elevation (Cerro Campana and Chiriqui) the stations

TABLE 1

Location and collecting periods at forest mosquito catching stations during 1950

Location	Province	Altitude (in feet)	Inclusive dates	Number of stations	Number of man days collecting		
					Ground	Canopy	Total
Panama							
El Real	Darien	50	3 July to 10 Aug.	4	20	20	40
Candelaria	Colon	200	14 Aug. to 22 Aug.	1	0	8	8
Cacique	Colon	200	19 Oct. to 30 Oct.	1	10	8	18
La Victoria	Panama	400–1,200	7 Feb. to 28 Dec.	2	87	88	175
Panama Viejo	Panama	10	6 July to 21 Dec.	1	17	0	17
Arraijan	Panama	600–800	5 June to 29 Dec.	6	49	103	152
Campanita Abajo	Panama	200	8 June to 30 Nov.	2	23	47	70
Campanita Arriba	Panama	600	28 Aug. to 4 Dec.	2	13	28	41
Campana, 2,000′	Panama	2,000	14 June to 6 Dec.	2	24	50	74
Campana, 2,800′	Panama	2,800	5 June to 21 Nov.	4	28	52	80
La Arena	Veraguas	400	19 Sept. to 28 Sept.	2	10	10	20
Tucue	Cocle	1,000	12 June to 14 Dec.	4	102	101	203
Sante Fe	Veraguas	2,500	25 May to 15 Aug.	3	60	60	120
Chorcha	Chiriqui	300	12 June to 11 Dec.	2	22	43	65
Progreso	Chiriqui	400	16 June to 14 Dec.	2	23	42	65
Bijao	Chiriqui	3,500	13 June to 12 Dec.	2	19	35	54
Palo Santo	Chiriqui	4,600	15 June to 1 Dec.	2	18	35	53
Costa Rica							
Esquinas	Puntarenas	300	6 June to 30 Nov.	4	90	87	177
Totals				46	615	817	1,432

were arranged to give a cross section of the forest fauna at different altitudes above sea level. The locations of the stations, their elevations, and the periods during which mosquitoes were collected at them are given in Table 1. In our previous study (Galindo, Trapido and Carpenter, 1950) collections had been made simultaneously of mosquitoes attacking human subjects on the forest floor, at a platform midway up a tree, and at a platform in the tree canopy. Since the middle platform did not produce species lacking in either the ground or canopy collections, the stations in the present survey were established with only one platform, in the canopy. In the 1949 study we had also operated each station for one half hour each hour from 8:00 A.M. to 5:30 P.M. To operate on this basis two crews of men had been necessary for each station, one for the morning and one for the afternoon. As the mid-day hours were generally the most productive of canopy mosquitoes, in the present study only one crew of men was used for each station, collecting continuously during the six hours from 9:00 A.M. to 3:00 P.M.

In general, each tree station was run one day each week for several months, but at certain of the localities such as Candelaria and Cacique which were difficult of access, and where it was only possible to send in a crew for a short

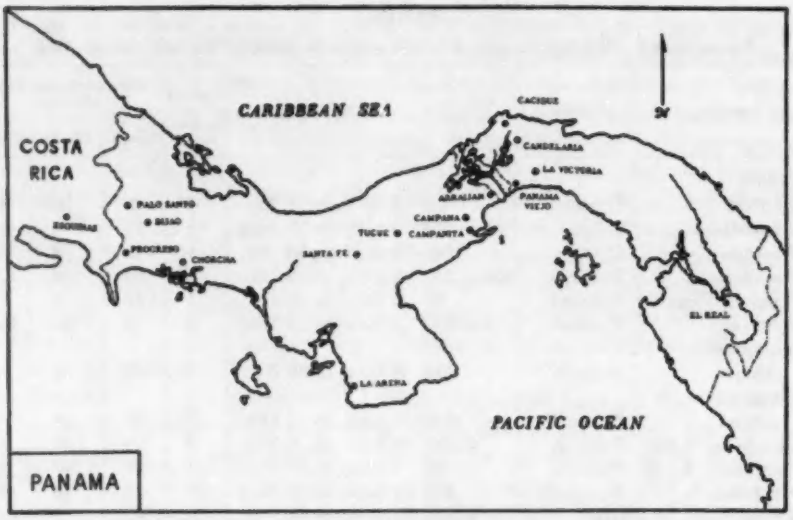


FIG. 1. Map of Panama showing locations of mosquito collecting stations

period, collections were made every day during a short period. In Table 1 are also given the number of man days of collecting, on the ground and in the canopy, at all stations. The geographic locations of the stations are shown in the map (Fig. 1). The method of making the collections has been described in a previous paper (Galindo, Trapido and Carpenter, 1950).

DISTRIBUTION OF SPECIES

In Table 2 are listed the 72 species of mosquitoes identified, arranged according to stations at which they were taken. In all, 72,211 mosquitoes were captured during 8,015 hours of collecting. Of these 31,530 were taken in 4,555 hours of collecting in the canopy and 40,681 in 3,460 hours on the ground.

The commonest and most widespread *Haemagogus* species was *equinus* which was taken at all stations, and made up more than half of all the individuals of this genus taken (6,488 *equinus* out of 11,848 *Haemagogus*). Only two specimens were taken in 80 hours of collecting at Panama Viejo, but this station was located in a coastal mangrove swamp and was intended only to establish densities of *H. chalcospilans*. The other stations at which very low numbers of this species appeared were at considerable elevation, Palo Santo being at 4,600 feet and Bijao at 3,500 feet. In the elevation cross section taken at Cerro Campana the highest density was found at the 2,000 foot level, with a fall off in numbers at 2,800 feet. From these data it is apparent that the species is common from near sea-level to 2,000 or 2,500 feet, becoming scarcer above this elevation, and quite rare above 4,000 feet. *Haemagogus lucifer* was also widespread in distribution from near the Colombian border to Costa Rica, but the total number taken was only about half that of *H. equinus*. At the Chiriqui stations and at Santa Fe

in Veraguas Province the species was very much less common than *H. equinus*, and at the most elevated station, Palo Santo, none were taken. *Haemagogus iridicolor*, which replaces *lucifer* to the west in Costa Rica, was rare and in all only seven specimens were collected, all at Bijao in Chiriqui Province. *Haemagogus spegazzinii falco* was of special interest since it is a proven vector of sylvan yellow fever in Colombia. The species was the least common of the widely distributed *Haemagogus*, comprising only 6.3 per cent of all the specimens of this genus taken. The only places at which substantial numbers were taken were La Victoria, where it had been found in our previous study, and Tucue in Cocle Province. Its abundance at Tucue might be explained by the fact that this was the only station in western Panama across the continental divide on the Caribbean slope, where the rainfall is higher, and true tropical rain forest better developed. Though none were taken at El Real, the station closest to Colombia, where the species is common, this may be explained by the fact that our station had to be located in second growth, as primary forest was too difficult to reach here. None were found at the 2,800 foot station at Cerro Campana, or the 4,600 foot station at Palo Santo, but there were a few at 3,500 feet at Bijao. It is also remarkable that at Santa Fe, where *H. equinus* was so common, an indication of the suitability of the area for tree hole *Haemagogus* breeding, none were taken. The small number of *spegazzinii falco* taken at Esquinas, Costa Rica (only five in 1,054 hours of collecting) has been interpreted by us (Galindo, Carpenter and Trapido, 1951a) as possibly due to the unsuitability of local conditions, since other *Haemagogus* also were not particularly common at this locality. The Esquinas area has a high and continuous rainfall which may not permit the intermittent drying and flooding of tree holes essential to hatching of *Haemagogus* eggs.

Thirteen species of *Aedes* of three subgenera were identified in the collections. The subgenus *Finlaya* was represented by the three species *leucocelaenus clarki*, *leucotaeniatus* and *terrens*. *Aedes leucocelaenus clarki* was found at stations from near the Colombian border into Costa Rica. More than half the specimens taken, 515 out of 975, were from the station at Tucue, across the divide on the Caribbean slope, which was also the station at which the bulk of the western Panama specimens of *H. spegazzinii falco* were taken. In Chiriqui this mosquito was taken in only very small numbers, but there were a few (five) found at the highest station at Palo Santo. At Cerro Campana they were commonest at 600 to 2,000 feet, and only a few were taken at 2,800 feet. As was the case in our previous study at Cerro La Victoria the total number of *leucocelaenus clarki* (975) approximated that of *H. spegazzinii falco* (750) and was much less than that of the other two *Haemagogus*, *equinus* with 6,488 and *lucifer* with 3,398. *Aedes leucocelaenus* and *H. spegazzinii* are proven vectors of sylvan yellow fever in South America. It is difficult to imagine their being able to maintain the disease on the Pacific slope of western Panama, should it be true that they are also the only vectors in Panama. The relatively high densities of these two species taken at the Tucue station might indicate that the disease could only pass through western Panama on the Caribbean side. The review by Elton (1952) of the location and sequence of

TABLE 2

Summary of diurnal forest mosquito catches during 1950

Station Location	El Real	Candelaria	Cacique	La Victoria	Panama Viejo	Arraijan	Campanita Abajo	Campanita Arriba	Campana 2000'	Campana 2800'	La Arena	Tucue	Santa Fe	Chorcha	Progreso	Bijao	Palo Santo	Esquinas Costa Rica	Total
Number of Hours	235	39	53	927	80	853	404	220	412	447	80	1196	719	360	378	279	279	1054	8015
Tribe Culicini																			
Haemagogus equinus	42	55	44	438	2	865	496	352	930	106	49	1387	1337	147	162	11	2	63	6488
Haemagogus lucifer	85	24	222	464		670	142	131	391	168	10	984	39	45	23				3398
Haemagogus iridicolor																7			7
Haemagogus lucifer—iridicolor																		119	119
Haemagogus spegazzinii falco			6	113								605		12		9		5	750
Haemagogus chalcospilans					1015														1015
Haemagogus argyromeris							55			1		2							58
Haemagogus sp							3		2							8			13
Total *Haemagogus*	127	79	272	1015	1017	1535	696	483	1323	275	59	2978	1376	204	185	35	2	187	11848
Aedes (Finlaya) leucocelaenus clarki	9	9		84		94	4	44	59	6	93	515		13	37		5	3	975
Aedes (Finlaya) leucotaeniatus		1	3	36		52	3	14	132	32		146	5	3	4		3	1	435
Aedes (Finlaya) terrens		2		10		18	12	4	113	150		139	281	53	96	36	582	39	1535
Aedes (Howardina) allotecnon																	1		1
Aedes (Howardina) quadrivittatus						4	15	38	963	69		14	73	3	17	33	998		2227
Aedes (Howardina) septemstriatus		5		1		2			14	12	1	14		1				1	51
Aedes (Howardina) sexlineatus									14			2							16
Aedes (Ochlerotatus) hastatus														1	6			3	10
Aedes (Ochlerotatus) serratus—group	716			4		8	3					17	102	112	1375	22		110	2469
Aedes (Ochlerotatus) taeniorhynchus	6			82	17	264	49	2				6		1					427
Aedes (Ochlerotatus) angustivittatus	683			1			1		39			8	21	38	222	1		10	1024

Aedes (Ochlerotatus) scapularis	1						1		2	2			85	4					95
Aedes (Ochlerotatus) fulvus	796							1						2	71				870
Aedes sp.	25	3		2			3					124	2724	36	129	84	22	11	3163
Psorophora (Psorophora) cilipes	2																		2
Psorophora (Psorophora) lineata	1											7			1				9
Psorophora (Janthinosoma) champerico												70							70
Psorophora (Janthinosoma) ferox	1381	1		13	1	29	13	27	9	57		256	1858	243	1706	51	78	459	6182
Psorophora (Janthinosoma) lutzii	393			23		2				1		28	308	14	81	5		12	867
Psorophora (Grabhamia) cingulata	120	9		1								26	17	2	2		1	4	182
Psorophora sp.				6															6
Mansonia (Mansonia) titillans-indubitans	10			631	9	121	1	6	3	3		7		12	34	2	7	36	882
Mansonia (Rhyncotaenia) venezuelensis	13			10		75								18	9			172	297
Mansonia sp.						9													9
Culex (Culex) sp.	6	1		1								8			1			19	36
Culex (Melanoconion) menytes																		1	1
Culex (Melanoconion) sp.														3	3			11	17
Culex (Carrollia) secundus		1																	1
Culex (Carrollia) urichii												1							1
Culex (Microculex) jenningsi																		1	1
Culex (Isostomyia) sp.												2							2
Orthopodomyia fascipes												1							1
Total *Culicini*	4289	111	275	1920	1044	2213	801	619	2671	607	153	4369	6850	763	3979	269	1699	1080	33712
Tribe Sabethini																			
Trichoprosopon (Trichoprosopon) digitatum		16		2			3	1		1	29	20			4			11	87
Trichoprosopon (Shannoniana) schedocyclius																1			1
Trichoprosopon (Shannoniana) fluviatilis		1														26	16		43
Trichoprosopon (Ctenogoeldia) magnus	2			1		8	1	13	3	1	80	460	177	3687	2442	36	176	1170	8257
Trichoprosopon (Isogoeldia) espini	17						1							63				3	84
Trichoprosopon (Rhunchomyia) longipes	21	11	1	26			47	17	5	1	123	315	10	640	149	20	57	617	2060
Trichoprosopon (Rhunchomyia) cerqueirai												6		2					8
Trichoprosopon (Rhunchomyia) lampropus		4										3	1						8
Trichoprosopon (Rhunchomyia) leucopus		5										17	1					1	24

TABLE 2—*Continued*

Station Location	El Real	Candelaria	Cacique	La Victoria	Panama Viejo	Arraijan	Campanita Abajo	Campanita Arriba	Campana 2000'	Campana 2800'	La Arena	Tucue	Santa Fe	Chorcha	Progreso	Bijao	Palo Santo	Esquinas Costa Rica	Total
Tribe Sabethini—*Continued*																			
Trichoprosopon sp.				8		1						2		10				21	42
Wyeomyia (Wyeomyia) abebela																13	12		25
Wyeomyia (Wyeomyia) arthrostigma	14			4					1										19
Wyeomyia (Wyeomyia) celaenocephala													2		3				5
Wyeomyia (Wyeomyia) florestan		1																	1
Wyeomyia (Wyeomyia) mitchellii				2					1	4	10		9			49	24	8	107
Wyeomyia (Wyeomyia) scotinomus									1										1
Wyeomyia (Wyeomyia) sp.	132	1079	41	143	13	119	10	49	135	87	164	3167	4777	608	904	1712	638	909	14687
Wyeomyia (Dendromyia) chalcocephala										1				11	9			1	22
Wyeomyia (Dendromyia) circumcincta																		1	1
Wyeomyia (Dendromyia) clasoleuca				1									4						5
Wyeomyia (Dendromyia) jocosa													140	7		103	11		261
Wyeomyia (Dendromyia) melanocephala	21											1		1				13	36
Wyeomyia (Dendromyia) aporonoma	2			29		1			1	2			22	30		50	73	6	216
Wyeomyia (Dendromyia) prolepidis	1												3						4
Wyeomyia (Dendromyia) sp.	113	69	15	18	4	41	20	37	49	15	190	3160	841	712	480	442	230	509	6945
Wyeomyia (Davismyia) arborea													4			16			20
Limatus assuleptus		14		2								174		8		6		5	209
Limatus durhamii	1	8		9			2	6			7	33		38	3	2	5	14	128
Limatus sp.																		4	4
Sabethes (Sabethes) cyaneus	2	1		30		38	6	12	7	1	29	120	6	65	11		3	18	349
Sabethes (Sabethes) tarsopus	18	1		18		60	31	33	30	2	23	322	11	52	9		28	18	656
Sabethes (Sabethinus) undosus												2		2					4
Sabethes (Sabethoides) chloropterus	35	41	13	280		627	71	177	305	17	161	753	193	105	278	4	11	140	3211
Sabethes sp.				1															1
Total *Sabethini*	379	1251	70	574	17	895	192	345	538	132	816	8555	6201	6041	4292	2480	1284	3469	37531

Tribe Anophelini																			
Anopheles (Anopheles) apicimacula	11	2	18	2		12		3						7	12			161	228
Anopheles (Anopheles) eiseni		6		2					2	1		7	163	4	7	3	6	15	216
Anopheles (Anopheles) punctimacula					3	1									1			22	27
Anopheles (Stethomyia) kompi												6			2				8
Anopheles (Nyssorhynchus) albimanus	17	1			40	6		3						1				75	143
Anopheles (Nyssorhynchus) oswaldoi	3																		3
Anopheles (Nyssorhynchus) strodei	13				3	5								1				97	119
Anopheles (Nyssorhynchus) triannulatus	2																	5	7
Anopheles (Kerteszia) neivai		6		1					3	3			174					2	189
Anopheles (Lophopodomyia) squamifemur		1																	1
Anopheles sp.																		1	1
Chagasia bathanus						6						1	5	1	4			9	26
Total *Anophelini*	46	16	18	5	46	30		6	5	4		14	342	14	26	3	6	387	968
Total All Species	4714	1378	363	2499	1107	3138	993	970	3214	743	969	12938	13393	6818	8297	2752	2989	4936	72211

Serratus-group includes: *serratus*, *oligopistus* and *tormenter*.

recent yellow fever cases in Panama and Costa Rica would confirm this view that the virus passed through western Panama on the Caribbean slope, and was not maintained in Panama on the Pacific slope west of the Canal Zone.

Aedes leucotaeniatus also appears to be distributed throughout the length of Panama, and into Costa Rica although none were taken at the easternmost station at El Real, and only one was captured in 1,054 hours of collecting at Esquinas, Costa Rica. The total number taken was about half that of *leucocelaneus clarki*. The species was most common at Tucue and at the 2,000 foot level at Cerro Campana. Thirty-two specimens were taken at the high station at Cerro Campana and three at 4,600 feet at Palo Santo in Chiriqui. *Aedes terrens*, which had been the least common *Finlaya* in our previous study in central Panama, was found to be rare in eastern and central Panama, but west of Cerro Campana it was, in general, the commonest representative of this subgenus. The numbers taken in western Panama were sufficient to make this the most abundant *Finlaya* taken. Unlike the other *Finlaya*, *terrens* was most abundant at higher elevations. At both Cerro Campana and in Chiriqui Province, the greatest numbers were taken at the most elevated stations. More than a third (582 out of 1,535) of the *terrens* taken were from the 4,600 foot station at Palo Santo.

Four species of the aedine subgenus *Howardina* were taken. Only a single specimen of *allotecnon* was identified, and the numbers of *septemstriatus* and *sexlineatus* were also insignificant. *Aedes quadrivittatus* was not taken east of the Canal Zone, but appeared throughout western Panama. Like *A. terrens* it was most abundant at some elevation, the great bulk of the specimens being taken at the 2,000 foot level at Cerro Campana and at 4,600 feet at Palo Santo in Chiriqui. It was not taken at the low elevation station at Esquinas in Costa Rica.

Of the subgenus *Ochlerotatus* six representatives were taken, and there were probably present in the collections two other species, *Aedes oligopistus* and *tormenter* which were lumped with *serratus* in the routine identification work. The numbers of *Aedes hastatus* were insignificant, but *serratus* was common and widespread, although exhibiting a gap in the Cerro Campana area. The species was commonest at low elevations, with only seven specimens being taken at the 4,600 foot station at Palo Santo. The location of the stations for this study in forest usually remote from the sea did not favor the collection of the coastal tidal marsh breeder, *Aedes taeniorhynchus*, and relatively few specimens were taken. *Aedes angustivittatus* was taken primarily at low elevations. *Aedes fulvus* was abundant only at the El Real station near sea-level in easternmost Panama, while *scapularis* was uncommon, the bulk (85 out of 95) of the specimens being from Santa Fe in Veraguas Province.

Representatives of the three subgenera of the genus *Psorophora* were taken. *Psorophora* (*Psorophora*) *cilipes* and *lineata* were represented by only two and nine specimens respectively. *Psorophora* (*Janthinosoma*) *ferox* was one of the commonest species of all, and was found throughout the length of the country. Relatively few, in terms of the total number taken, were at high elevations. *Psorophora* (*Janthinosoma*) *lutzii* was also generally distributed, but much less

abundant. The third *Janthinosoma*, *champerico*, was taken only at the station at Tucue, although in the previous study a few were found at La Victoria. *Psorophora* (*Grabhamia*) *cingulata* was found in small numbers. All were at low elevations except a single specimen taken at the highest station at Palo Santo.

Three species of the genus *Mansonia*, i.e., *titillans*, *indubitans*, and *venezuelensis* were taken. The species *titillans* and *indubitans* were not separated in the routine identification work and they are recorded together in the table.

The genus *Culex* was represented by the subgenera *Culex*, *Melanoconion*, *Carrollia*, *Microculex* and *Isostomyia*, but only insignificant numbers were taken approaching man during the day in the forest. By contrast very great numbers of a large diversity of species may be taken at light traps operated at night, and several species may be taken biting man at night in the forest.

Only a single specimen of *Orthopodomyia fascipes* was collected.

The genus *Trichoprosopon* was represented in the collections by nine species of five subgenera. The most common species was *T.* (*Ctenogoeldia*) *magnus* of which 8,257 specimens were taken. The great bulk of these were from Cocle, Veraguas and Chiriqui Provinces in western Panama and Puntarenas Province in Costa Rica. In this western area it was most abundant at the low elevation stations near extensive stands of *Calathea* in which it breeds. Next in abundance was *T.* (*Rhuncomyia*) *longipes*, of which 2,060 specimens were taken. This species was widespread, having been taken at 16 of the 18 stations. As was the case with *T. magnus*, it was most abundant at the stations in western Panama and adjacent Costa Rica, at low elevations. *Trichoprosopon carqueìraì*, *lampropus* and *leucopus*, three other species of the subgenus (*Rhuncomyia*), were rare. Small numbers of *T.* (*Trichoprosopon*) *digitatum* were found distributed from Candelaria in eastern Panama, west into Costa Rica. The subgenus *Shannoniana* was represented by two species, *schedocyclius* and *fluviatilis*, both of which were rare. *Trichoprosopon* (*Isogoeldia*) *espini* was taken in only small numbers at four of the 18 localities, but was present at the easternmost at El Real and the westernmost in Costa Rica.

The genus *Wyeomyia* made up almost a third (22,355) of all the mosquitoes taken. The identification of females of this genus, particularly when specimens have the scales rubbed, is difficult or impossible, so that the great bulk of the material was determined only to subgenus. The relatively small number on which species identifications were made, does not permit generalization about their distribution. On the subgenus *Wyeomyia* six species were identified as follows: *abebela*, *arthrostigma*, *celaenocephala*, *florestan*, *mitchellii*, and *scotinomus*. Seven species of the subgenus *Dendromyia* were determined: *chalcocephala*, *circumcincta*, *clasoleuca*, *jocosa*, *melanocephala*, *aporonoma*, and *prolepidis*. In the course of the study a representative of the South American subgenus *Davismyia* was found and described as a new species, *arborea* (Galindo, Carpenter and Trapido, 1951).

The genus *Limatus* was represented by the two species *assuleptus* and *durhamii*, of which only small numbers were taken.

Sabethes (*Sabethes*) *cyaneus* and *tarsopus* were found to be widely distributed in modest numbers throughout the length of the Republic and into Costa Rica, with

tarsopus being about twice as abundant as *cyaneus*. Both species were present at the highest station at Palo Santo. *Sabethes* (*Sabethinus*) *undosus*, was very rare, with only four specimens taken. *Sabethes* (*Sabethoides*) *chloropterus* was the most common sabethine found in our previous study in central Panama. In the present study it was also common (3,211 specimens) but was outnumbered by one other sabethine, *Trichoprosopon magnus* (8,257 specimens). The only station at which *chloropterus* was not taken was Panama Viejo, which was located by a coastal mangrove swamp in which it would not be expected. The species was more common at stations below 2,000 feet than those higher.

The anophelines were not of special interest in this study of diurnal forest mosquitoes but ten species were taken of the genus *Anopheles* and one *Chagasia*. The species collected were the following: of the subgenus *Anopheles*, the species *apicimacula*, *eiseni* and *punctimacula*; of the subgenus *Stethomyia*, the species *kompi*; of *Nyssorhynchus*, the species *albimanus*, *oswaldoi*, *strodei* and *triannulatus*; of *Kerteszia*, the species *neivai*; and of *Lophopodomyia* the species *squamifemur*. Most common were *apicimacula* and *eiseni*, followed in numbers by *neivai* and *albimanus*.

VERTICAL STRATIFICATION

It is now well known that forest mosquitoes show a marked stratification in their vertical distribution. This stratification is most marked in the true tropical rain forest where the tree canopy is dense and the forest floor is deeply shaded and protected from the effects of wind on temperature and humidity. In the more open deciduous tropical forest, characteristic of areas with lower rainfall and a severe dry season, the difference between the microclimate of the canopy and the forest floor is less marked. This results in a less distinct stratification of the mosquito fauna as well. In the present study the stations at which collections were made were by necessity located in the deciduous tropical forest of the Pacific slope, with the exception of that at Tucue which was just across the continental divide in the higher rainfall Atlantic drainage. Thus the data in Table 3 and Figure 2 showing the proportion of each species taken on the ground and in the canopy indicate a less well marked stratification than might be found in true tropical rain forest.

Only species of which 15 or more specimens were taken are included in the table and chart. The percentages of each species taken on the ground and in the canopy are corrected for the numbers of man hours during which the collections were made, and only the numbers of man hours of collecting at the stations at which the species were actually taken were used in the calculations. An examination of the histogram in Figure 2 will show that most of the species of mosquitoes may readily be classified as attacking primarily either at ground level or in the canopy with relatively few being indiscriminate in this regard. More than half the species (25 out of 47) had 20 per cent or less of their numbers taken in the canopy while an additional eight species had 21 to 40 per cent attacking in the canopy. The number of arboreal species is smaller, with only six species being 81 to 100 per cent in the canopy and six falling between 61 and 80 per cent. Only two

TABLE 3

The vertical distribution of diurnal forest mosquitoes

Species	Per cent captured		Number taken
	Canopy	Ground	
Haemogogus equinus	69.1	30.9	6,431
Haemagogus lucifer	67.1	32.9	3,351
Haemagogus spegazzinii falco	80.7	19.3	743
Haemagogus argyromeris	9.7	90.3	58
Aedes leucocelaenus clarki	65.7	34.3	966
Aedes leucotaeniatus	40.1	59.9	434
Aedes terrens	17.4	82.6	1,533
Aedes quadrivittatus	74.3	25.7	2,227
Aedes septemstriatus	14.3	85.7	46
Aedes sexlineatus	10.9	89.1	16
Aedes serratus (group)	9.8	90.2	2,476
Aedes taeniorhynchus	2.0	98.0	632
Aedes angustivittatus	16.7	83.3	804
Aedes scapularis	13.1	86.9	95
Aedes fulvus	10.4	89.6	883
Psorophora champerico	1.5	98.5	70
Psorophora ferox	23.2	76.8	6,180
Psorophora lutzii	23.0	77.0	867
Psorophora cingulata	19.7	80.3	173
Mansonia titillans-indubitans	8.7	91.3	877
Mansonia venezuelensis	24.1	75.9	293
Trichoprosopon digitatum	15.9	84.1	71
Trichoprosopon fluviatilis	11.0	89.0	42
Trichoprosopon magnus	73.0	27.0	8,257
Trichoprosopon espini	60.1	39.9	84
Trichoprosopon longipes	20.8	79.2	2,049
Trichoprosopon leucopus	0.0	100.0	19
Wyeomyia abebela	36.2	63.8	25
Wyeomyia arthrostigma	81.7	18.3	19
Wyeomyia mitchellii	10.1	89.9	107
Wyeomyia chalcocephala	0.0	100.0	21
Wyeomyia jocosa	28.3	71.7	261
Wyeomyia melanocephala	48.6	51.4	36
Wyeomyia aporonoma	2.8	97.2	216
Wyeomyia arborea	100.0	0.0	20
Limatus assuleptus	2.8	97.2	195
Limatus durhamii	14.6	85.4	120
Sabethes cyaneus	87.4	12.6	348
Sabethes tarsopus	87.4	12.6	655
Sabethes chloropterus	75.9	24.1	3,170
Anopheles apicimacula	10.3	89.7	226
Anopheles eiseni	18.1	81.9	210
Anopheles punctimacula	0.0	100.0	24
Anopheles albimanus	8.5	91.5	102
Anopheles strodei	7.1	92.9	116
Anopheles neivai	40.3	59.7	183
Chagasia bathanus	85.8	14.2	26
Total			45,757

THE VERTICAL DISTRIBUTION OF DIURNAL FOREST MOSQUITOES

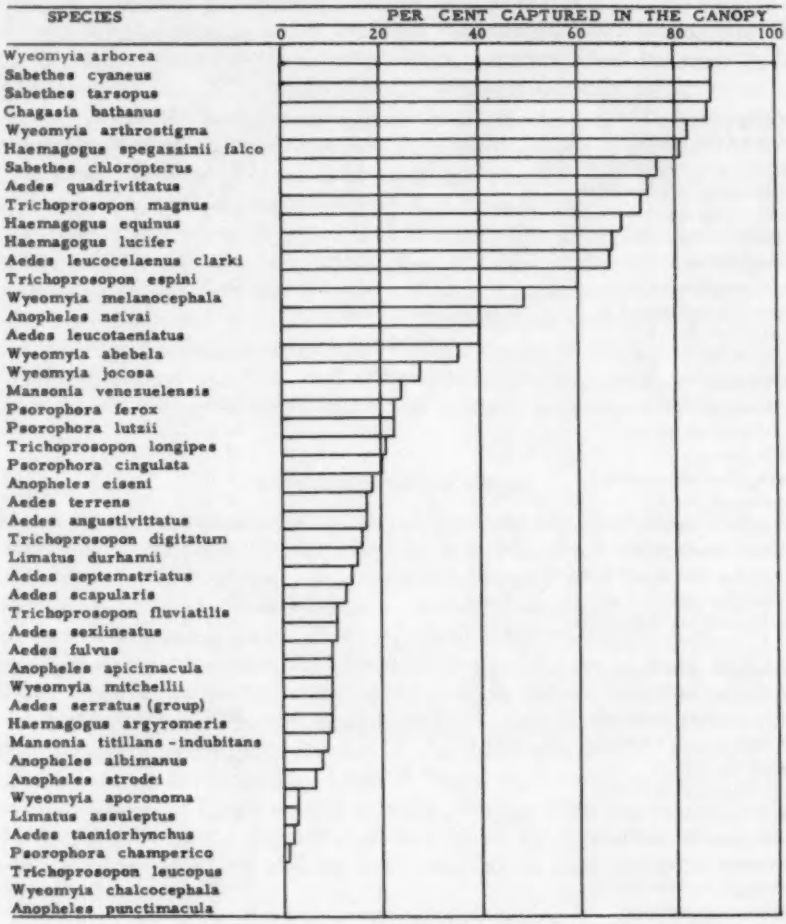


FIG. 2

species appear intermediate with 40 to 60 per cent arboreal. Three species, of which relatively few specimens were taken, were confined completely to the ground level, while one species, the recently described *Wyeomyia arborea*, was taken only in the canopy.

As in our previous study we found that of the *Haemagogus*, the species *spegazzinii falco* was the most arboreal, with 80.7 per cent being taken in the canopy. *Haemagogus argyromeris*, of which very few were taken except in the low, dry, open forest at Campanita Abajo, was the only *Haemagogus* which was not arboreal, with 90.3 per cent being taken on the ground.

Of all the species of *Aedes* the most arboreal was *quadrivittatus*, with 74.3 per

cent taken in the canopy, while the other two representatives of the subgenus *Howardina*, *septemstriatus* and *sexlineatus*, were taken primarily on the ground. The three species of the subgenus *Finlaya* were found to be vertically stratified in the same order as in our previous study, with *terrens* being primarily terrestrial, *leucocelaenus clarki* the most arboreal and *leucotaeniatus* intermediate. The *Ochlerotatus* species all were most abundant on the ground.

The *Psorophora* and *Mansonia* also were taken in greatest numbers on the ground.

The most arboreal of the *Trichoprosopon* was the species *magnus* with 73.0 per cent taken in the canopy followed by *espini* with 60.1 per cent in the tree tops. The remaining species taken, *digitatum*, *fluviatilis*, *longipes* and *leucopus* were most abundant on the ground.

Only a very small number of the very numerous *Wyeomyia* were identified to species. The species *arborea*, discovered and described in the course of this study (Galindo, Carpenter and Trapido, 1951) was the one species taken in the study entirely in the canopy. Predominantly arboreal also was the species *arthrostigma* with 81.7 in the canopy. The remaining species had more than 50 per cent of their numbers taken on the ground, but the percentages given in the table and chart would be subject to revision if larger numbers had been identified to species.

The two species of the genus *Limatus* were most abundant on the ground.

The figures for the *Sabethes* are somewhat at variance with those obtained in our previous study. In the present study *cyaneus* and *tarsopus* were equally abundant in the canopy (87.4 per cent) with *chloropterus* somewhat less so (75.9 per cent). In our earlier study the positions of *cyaneus* and *chloropterus* were reversed with *chloropterus* appearing to be more arboreal than *cyaneus*.

As the present collections were made during the daylight hours the number of anophelines taken was not large. All the species of the genus *Anopheles* were either completely or primarily taken on the ground with the exception of the epiphytic plant breeding representative of the subgenus *Kerteszia*, *neivai*, of which 40.3 per cent were in the canopy. As was found in our previous study, *Chagasia bathanus* was taken most often in the canopy, with but 14.2 per cent of the catch on the ground.

DISCUSSION AND CONCLUSIONS

It is evident from the preceding review of forest mosquito collections made throughout the length of Panama and into Costa Rica, that there is no consistent shift in the composition of this fauna which might be used in explanation of the failure of sylvan yellow fever to appear west of the Canal Zone prior to 1950. The known vector species in South America, *Haemagogus spegazzinii falco* and *Aedes leucocelaenus clarki* were found in western Panama and Costa Rica. The other species of *Haemagogus*, the *Aedes* of the subgenus *Finlaya*, and the *Sabethes* which might be considered suspect as vectors on epidemiological grounds, all continue their ranges through the Isthmian region, with the exception of *Haemagogus lucifer* which is replaced in westernmost Panama and Costa Rica by *H. iridicolor*. The significant positive finding for the area under study is that the

mosquitoes which are the known South American vectors of yellow fever and their ecological associates were only abundant at the Atlantic drainage station at Tucue, where the forest is most representative of the true tropical rain forest type of the Amazon and Orinoco basin in which sylvan yellow fever permanently persists. Related to this fact is the circumstance that the proven yellow fever fatalities west of the Canal Zone in Panama during 1950 and 1951 (Elton, 1952) were contracted in this Atlantic slope rain forest, while yellow fever failed to appear in the western provinces of the Pacific side. The critical factor would thus appear to be not geographical but ecological.

In our introduction we mentioned the rainfall differential between the Atlantic and the Pacific slopes of the Isthmus, and the consequent difference in forest type in the two drainages. We might add that as a result of the gap in the mountain system in the Canal Zone area, the northeasterly trade winds here spill across the Isthmus to the Pacific side, so that just east of the Canal Zone in the La Victoria area and just west of the Zone at Arraijan, the forest while not so well developed as the Atlantic side rain forest, is at least intermediate between rain forest and deciduous forest. When we review the data presented here we find that the known South American vectors and their ecological associates were most abundant in the best developed rain forest, at Tucue, secondary in numbers in the transitional forest at such places as La Victoria and Arraijan, and sparse, rare, or wholly absent in deciduous or scrub forest at such places as Campanita Abajo and Arriba which are in the rain shadow of Cerro Campana.

This leaves us still with the question of why, if the immunity surveys conducted in Panama, Central America and Mexico (cited above) are taken at face value, sylvan yellow fever was confined in the past decades to the area of Panama east of the Canal Zone, contiguous with South America. The data presented here for the Pacific slope of western Panama are ample to show the incidence of known vectors to be too low to be considered capable of maintaining virus. But what of the Atlantic slope forest and the fact that sylvan yellow fever did finally pass through this area in the period 1949 to 1951? The explanation would be this. The gap in the mountain system in the Canal Zone area, as mentioned above, results in a spilling of moisture onto the Pacific slope which would otherwise fall in the Atlantic drainage. This is inadequate in amount and annual distribution to produce true rain forest on the Pacific slope, but also deprives the Atlantic side of the moisture necessary to maintain this ecological situation. The conditions for the vector species, *Haemagogus spegazzinii falco* and *Aedes leucocelaenus clarki*, are in consequence only marginal in the area. The ability of the virus to cross this marginal zone would thus depend on the coincidence of the approach of the virus from the east with a year of particularly favorable rainfall conditions which would result in an optimum production of the vector species at the critical time. This interpretation would gain substance if it could be demonstrated that there are long term as well as annual cycles of abundance of these mosquitoes. That such long term cycles, which are well known among anophelines, also occur in forest dwelling, tree hole breeding mosquitoes such as *Haemagogus* and *Aedes* (*Finlaya*) has been suggested by Causey and Dos Santos (1949) in Brazil.

Subsequent to the studies reported here, the observations at the La Victoria stations were continued, so that we now have data on the abundance cycle of these mosquitoes over a period of years. These data will be the subject of a separate paper, but it may be said now that they do support the hypothesis advanced here.

SUMMARY

Collections of forest mosquitoes totaling 72,211 individuals were made on the ground and in the canopy during the rainy season of 1950 at 18 localities throughout the length of Panama and into Costa Rica, in seeking an explanation for the failure of sylvan yellow fever to appear west of the Canal Zone prior to that time.

It was found that the known South American vectors of the disease, *Haemagogus spegazzinii falco* and *Aedes leucocelaenus clarki* occurred sparingly west into Costa Rica, but the only station at which they were present in substantial numbers was one located in the well developed tropical rain forest of the Atlantic slope.

The vertical stratification of the mosquitoes taken in the forest is summarized for each species of which more than 15 individuals were taken. These data are of importance in assessing the possible role of each species in terms of its contact with the arboreal mammals which are the intermediate hosts of the yellow fever virus.

It is suggested that physiographic and meteorologic conditions in central Panama and the Canal Zone area produce a forest cover which is intermediate between tropical rain forest favorable for the production of substantial numbers of tree hole breeding vector mosquitoes, and tropical deciduous forest in which conditions are less favorable. It is further suggested that since conditions are marginal in this critical area, it would take a particularly favorable rainfall pattern to produce the mosquito population necessary to carry the virus across into the Atlantic side rain forest of western Panama as happened in 1949 and 1950.

LITERATURE CITED

Bustamante, M. E., Kumm, H. W., and Herrera, J. R., 1942. Ausencia de fiebre amarilla en el valle del Usumacinta, *Rev. Inst. Sal. Enf. Trop.* **3**: 255–271.

Causey, O. R., and dos Santos, G. V., 1949. Diurnal mosquitoes in an area of small residual forests in Brazil, *Ann. Ent. Soc. Amer.* **42**: 471–482.

Clark, H. C., 1938. Annual report of the Gorgas Memorial Laboratory, 1937: 1–24.

Elton, N. W., 1952. Sylvan yellow fever in Central America, *Pub. Health Repts.* **67**: 426–432.

Galindo, P., Carpenter, S. J., and Trapido, H., 1951. Description of two new species of *Wyeomyia* and the male of *Sabethes tarsopus* Dyar and Knab. *Proc. Ent. Soc. Wash.* **53**: 86–96.

Galindo, P., Carpenter, S. J., and Trapido, H., 1951a. Westward extension of the range of *Haemagogus spegazzinii falco* Kumm *et al.* into Costa Rica, *Proc. Ent. Soc. Wash.* **53**: 104–106.

Galindo, P., Trapido, H., and Carpenter, S. J., 1950. Observations on diurnal forest mosquitoes in relation to sylvan yellow fever in Panama, *Am. J. Trop. Med.* **30**: 533–574.

HERRERA, J. M., ELTON, N. W., AND NICOSIA, J., 1949. La aparición en Panamá de un brote de fiebre amarilla en su forma "selvática" (1948–1949) descubierto por el examen postmortem, *Arch. Hosp. Santo Tomas* **4**: 1–49.

KUMM, H. W., AND CRAWFORD, P. J., 1943. The recent distribution of endemic yellow fever in Central America and neighboring countries, *Am. J. Trop. Med.* **23**: 421–431.

SAWYER, W. A., 1942. The yellow fever situation in the Americas, *Proc. 8th Sci. Cong.* **6**: 297–312.

SAWYER, W. A., BAUER, J. H., AND WHITMAN, L., 1937. The distribution of yellow fever immunity in North America, Central America, the West Indies, Europe, Asia, and Australia, with special reference to the specificity of the protection test, *Am. J. Trop. Med.* **17**: 137–161.

FOREST CANOPY MOSQUITOES ASSOCIATED WITH THE APPEARANCE OF SYLVAN YELLOW FEVER IN COSTA RICA, 1951

PEDRO GALINDO AND HAROLD TRAPIDO

Gorgas Memorial Laboratory, Panama, R. de P.

During 1951 yellow fever appeared in the Atlantic drainage of Costa Rica, producing a widespread epizootic in monkeys and a number of human cases with some fatalities (Elton, 1952; Vargas and Elton, 1953). Following the first recognized human fatality contracted near Puerto Limon, the authors were invited by Dr. Oscar Vargas Mendez, Director General of Public Health of Costa Rica, to undertake investigations of the mosquito fauna associated with sylvan yellow fever in the area. An attempt was also to be made to establish the vector or vectors by isolation of virus from mosquitoes caught in the vicinity of proven yellow fever fatalities.

A field investigation of this first human case revealed that the victim had been supervising a crew of men thinning out the forest trees used as shade at a cacao plantation on a tract of land known as Wauchope, about 10 miles southwest by narrow gauge rail from Puerto Limon. Two local woodsmen were engaged to build rough ladders into the canopy of trees bordering the cacao plantation, and to collect alive in shell vials such blue mosquitoes as attempted to bite them during the daylight hours they were stationed in the trees. These men were put under the daily supervision of a local sanitary inspector kindly made available by Dr. Vargas. By instructing the men to catch only "blue mosquitoes" we restricted the collections almost entirely to mosquitoes of the genus *Haemagogus* *Aedes* of the subgenus *Finlaya*, and certain of the sabethines. Thus we eliminated from the collections virtually all of the ground breeding mosquitoes such as *Psorophora*, *Mansonia*, and *Aedes* of the subgenus *Ochlerotatus* in which we were not interested. The human fatality contracted at Wauchope had occurred on July 24th. The infection had presumably been contracted about a week previous. As the work at Wauchope was begun on August 9th, we were approximately three and a half weeks late in starting the collecting. Mosquito captures were continued here through September 13th.

Since we were so late in getting started at Wauchope, we attempted to estimate by projection where yellow fever might be expected during the following month so that we might have additional mosquito collecting stations located where yellow fever was concurrently active. This estimate was based on our experience with the ecological preferences of *Haemagogus spegazzinii falco* and *Aedes leucocelaenus clarki*, two known South American vectors of yellow fever which we had found present at the locations where yellow fever had appeared in Panama the previous two years (Galindo *et al.*, 1950; Trapido *et al.*, 1955). Thus we sought a location supporting true tropical rain forest on slopes 100 to 500 meters above sea level, if possible within 50 or 75 miles of the already known focus. Another consideration was that of selecting a site from which the collections of living

FIG. 1 (Upper). San Miguel, Costa Rica. View of the rolling hill country of the basin of the Rio Sarapiqui from the base of the Cordillera Central. The rain forest here is only partially disturbed by cultivation.

FIG. 2 (Lower). Collecting *Haemagogus* at ground level in cacao plantation at Wauchope. The broken canopy of the original rain forest permits the filtering through to the ground level of the microclimatic conditions of the upper stories of the forest.

mosquitoes could be gotten out for shipment by air on a daily basis to our laboratory in Panama. The second locality chosen for these reasons was in the vicinity of San Gerardo. This settlement was located in the basin of the Rio Sarapiqui, seven hours by horse from San Miguel, in the rolling hill country at the northern

foot of the Cordillera Central (see Figure 1). The elevation was about 250 meters. During the reconnaissance of this area on August 11th and 12th local persons told us of monkey mortality, and San Gerardo was specifically chosen as a collecting site when a woodsman took us to the decomposed carcass of a spider monkey (*Ateles*). Subsequently, human cases of yellow fever contracted in this area were diagnosed. The collections were begun here on August 13th and continued until September 18th. At this locality four men were employed to make the collections in the same manner as at Wauchope.

During the weeks following the establishment of these collecting stations, various delays were experienced in getting the mosquitoes to Panama. We found it took from three to 11 days for the mosquitoes to reach us, and as much as 80 or 90 per cent mortality was experienced, although the individual vials in which the mosquitoes were captured and shipped were provided with cotton plugs moistened in sugar solution. While the attempts at virus recovery were thus handicapped, and in fact results were negative, a good sample was obtained of the forest canopy mosquito fauna associated with sylvan yellow fever in this newly invaded area.

DISCUSSION OF RESULTS

A summary of the mosquitoes taken is given in Table 1. The literature covering the present knowledge of the status of various mosquitoes as vectors of yellow fever has recently been summarized by Whitman (1951), and for the sake of brevity the original references will not be individually cited here. They may be found in Whitman.

Of greatest interest are the collections of *Haemagogus*, the genus widely implicated in the transmission of sylvan yellow fever in South America. The most abundant *Haemagogus* at Wauchope was *H. spegazzinii falco*, a species from which yellow fever virus has repeatedly been isolated in Colombia. The species was at this place more abundant than we had found it at the Panama localities where yellow fever appeared (Galindo *et al.*, 1950). At San Gerardo it was less common, but even so the numbers were greater than those taken at the same time of year at the Panama stations. While it is always possible that there are sylvan vectors of yellow fever about which we do not know, the association of *spegazzinii falco* with yellow fever in this case may best be taken at face value.

In Panama it had been our experience that *spegazzinii falco* was a mosquito of primary tropical rain forest, and that it was absent or rare in second growth, or in areas of relatively low rainfall which supported only deciduous tropical forest. The species is also strongly arboreal. It was thus with some surprise that we found in our initial survey at Wauchope that *spegazzinii falco* was attacking in some numbers on the ground in the cacao plantation which was only partially shaded by the residue of forest trees. This experience was repeated when we returned to Wauchope on a short visit in 1954. It would seem that in areas of abundant rainfall where the shade utilized for the growing of cacao is obtained by only partially clearing the original rain forest cover, the conditions of light, temperature and humidity which comprise the microclimate of the forest canopy

TABLE 1

Forest canopy mosquito collections from Costa Rica, 1951

	San Gerardo Alajuela Prov. 9 Aug. to 13 Sept.	Wauchope. Limon Prov. 13 Aug. to 18 Sept.	Total
Haemagogus equinus	24	123	147
Haemagogus lucifer—iridicolor	331	157	488
Haemagogus spegazzinii falco	96	331	427
Total Haemagogus	451	611	1,062
Aedes leucocelaenus	5	2	7
Aedes leucotaeniatus	3		3
Aedes fulvus	1		1
Aedes serratus	1		1
Aedes angustivittatus	2		2
Psorophora ferox	12	2	14
Psorophora lutzii	3		3
Psorophora sp.	1		1
Total Culicini	479	615	1,094
Wyeomyia (*Wyeomyia*)	15	4	19
Wyeomyia (*Dendromyia*)	21	13	34
Sabethes cyaneus	26	39	65
Sabethes tarsopus	17	24	41
Sabethoides chloropterus	86	111	197
Total Sabethini	165	191	356
Anopheles neivai	2		2
Total Anophelini	2		2
Total all mosquitoes	646	806	1,452

are similated at ground level, and the vertical distribution of such mosquitoes as *spegazzinii falco* is thereby modified (See Figure 2 and 3). A similar situation where forest trees have been permitted to remain as shade for coffee is reported by Bugher *et al.* (1944). In some places the shade desirable for the propagation of coffee and cacao is obtained by artificial plantings of shade trees after complete clearing. Under these conditions *Haemagogus spegazzinii falco* would not be expected, probably at least in part because there has not been adequate time for the appearance of rot holes in the trees necessary for larval development. The significance of this modification of the forest cover which brings the microclimate of the canopy down to ground level is that in such situations there is danger of contracting sylvan yellow fever on the ground. In undisturbed primary rain forest, the ecological conditions in the canopy are so different from those on the ground, that persons may pass with relative safety through such forest without

FIG. 3. Mosquito collecting station at Wauchope, Costa Rica. The original primary tropical rain forest has been thinned by selective cutting, permitting the sunlight to reach the ground level which is utilized as cacao plantation. Under these conditions arboreal mosquitoes come down to ground level.

being exposed to biting by canopy mosquitoes which may have become infected from feeding on arboreal primates.

Haemagogus equinus, which had been the commonest species of the genus found in the Panama studies, was the least common *Haemagogus* at the Costa Rican stations. This species is known to be capable of transmitting yellow fever in the laboratory, but virus has never been recovered from it with certainty in nature. As it was outnumbered by *spegazzinii falco* at both localities studied, we have no need to hypothesize its involvement in the transmission at these places.

Haemagogus iridicolor replaces *Haemagogus lucifer* in western Panama and Costa Rica, but as the two are not readily separable as females they are recorded together, although the collections probably represent only *iridicolor*. These slightly outnumbered *spegazzinii falco* in the collections. There is no information available on the ability of either of these species to transmit yellow fever.

Another proven vector of sylvan yellow fever in nature is *Aedes leucocelaenus clarki*. In the present collections it was so rare, only seven individuals being taken in all, that it may be excluded from consideration. Significant numbers of other *Aedes* and *Psorophora* were not taken in the tree canopy as the collectors concentrated on capturing "blue mosquitoes."

The commonest sabethine mosquito taken was *Sabethes chloropterus*. We have suspected that this species might be involved in sylvan yellow fever transmission in Panama on the basis of its habits, distribution, association with the appearance of yellow fever, and the fact that there is some partial evidence in support of its ability to carry virus. In Panama it was generally present in about the same numbers as *spegazzinii falco*, but differed in that captures were made throughout the dry season when *spegazzinii falco* could not be found. In the present case it is not necessary to hypothesize its involvement since such substantial numbers of *spegazzinii falco* were present, although we consider it of interest that once again it can be shown to be associated with the presence of sylvan yellow fever. The other sabethines were present in lesser numbers and appear to be of no special interest.

SUMMARY AND CONCLUSIONS

During August and September 1951, forest canopy mosquito collections were made at two localities in Costa Rica where sylvan yellow fever was active. *Haemagogus spegazzinii falco*, a species which has been found naturally infected with yellow fever on a number of occasions in Colombia, was the commonest species of the genus present at Wauchope and was at least as abundant at San Gerardo as it had been at localities where yellow fever appeared in Panama in the two years previous. The conservative course would be to take this association at face value and consider this species the probable vector in this outbreak.

Haemagogus spegazzinii falco is ordinarily an inhabitant of the upper stories of primary tropical rain forest, but at Wauchope we found it commonly at ground level in cacao plantation in which the original forest cover had been thinned to provide the partial shade necessary for cacao trees. In these circumstances the canopy habitat is simulated at ground level and the stratification of

spegazzinii falco is less pronounced. Such a situation provides conditions under which there is danger of sylvan yellow fever transmission on the ground.

Other *Haemagogus* present were *H. equinus* and *H. lucifer-iridicolor*. The former is known to be capable of transmitting virus in the laboratory but has never been found infected with certainty in nature. Nothing is known of the vector status of the latter two species. As the numbers of *H. spegazzinii falco* present were ample to explain the transmission of yellow fever in the area, these species need not be considered in the present circumstance.

The commonest sabethine mosquito present was *Sabethes chloropterus*. Its status as a vector is unknown, but on epidemiological grounds we considered it suspect in Panama, and note with interest its present association with yellow fever.

REFERENCES

Bugher, J. C., Boshell-Manrique, Jorge, Roca-Garcia, Manuel, and Osorno-Mesa, Ernesto, 1944. Epidemiology of jungle yellow fever in Eastern Colombia, *Am. J. Hyg.* **39**: 16–51.

Elton, N. W., 1952. Sylvan yellow fever in Central America, *Pub. Hlth. Repts.* **67**: 426–432.

Galindo, Pedro, Trapido, Harold, and Carpenter, S. J., 1950. Observations on diurnal forest mosquitoes in relation to sylvan yellow fever in Panama, *Am. J. Trop. Med.* **30**: 533–574.

Trapido, Harold, Galindo, Pedro, and Carpenter, S. J., 1955. A survey of forest mosquitoes in relation to sylvan yellow fever in the Panama Isthmian area, *Am. J. Trop. Med. Hyg.* **4**:

Vargas-Mendez, Oscar, and Elton, N. W., 1953. Naturally acquired yellow fever in wild monkeys of Costa Rica, *Am. J. Trop. Med. Hyg.* **2**: 850–863.

Whitman, L., 1951. The arthropod vectors of yellow fever. In *Yellow Fever*, G. K. Strode, Ed., McGraw-Hill Book Co., N. Y.: 229–298.

RABIES TRANSMITTED BY BATS IN BRITISH GUIANA

B. B. G. Nehaul

Central Medical Laboratory, Georgetown, British Guiana

The term "paralytic rabies" is here used for rabies transmitted by bats. The first human cases of paralytic rabies were reported in Trinidad in 1925. Hurst and Pawan (1932) reported rabies with the symptoms of acute ascending myelitis in man in Trinidad. In subsequent papers, Pawan gave a historical review of the disease and described the disease in man and in vampire bats. Up to the end of 1953, eighty-nine deaths had been registered in that island. In Mexico, in April, 1951, five cases were reported in the State of Sinaloa. In June, the same year, three persons died with clinical symptoms of paralytic rabies in the State of Jalisco. In May 1952, another case was reported in Ixtlan del Rio. Venters *et al.* (1954) reported rabies in insectivorous bats in Florida and gave an account of an attack on a boy by a bat subsequently shown to be suffering from rabies. Witte (1954) reported rabies in insectivorous bats in Pennsylvania and also gave an account of a woman having been attacked by a rabid bat.

Although paralytic rabies occurs among cattle in Brazil, Venezuela and Central America, human cases have not been reported in South America. This is an account of an outbreak of paralytic rabies in the Mazaruni District in British Guiana during the months of August and September, 1953, when nine persons died from the disease.

British Guiana lies on the mainland of South America. On the east is Dutch Guiana, on the west, Venezuela, on the south and south-west lies Brazil, and on the north the Atlantic Ocean.

THE OUTBREAK IN BRITISH GUIANA

The outbreak occurred among miners working on one bank of the Kurupung Creek near its mouth, where it flows into the Mazaruni River, a tributary of the Essequibo River. There were forty-three persons in the locality, half a mile along the bank of the creek and about half a mile inland. The region is a forest area and miners seek diamonds in shallow pits. Trees grow on the banks of rivers and creeks and overhang the water. The miners live in camps or in huts open on all sides. They sleep on roughly made cots or in hammocks and rarely use mosquito nets. Some sleep in sacks which they pull over their legs like a sleeping bag. Many are bitten by vampire bats. Very few keep a lamp burning at night.

On the eighteenth of September, 1953, two miners were brought to the government hospital at Georgetown. Both had been working together and both had similar symptoms and signs—the clinical features of an acute ascending myelitis. The first died without a definite diagnosis having been made before or after death. In the second, a clinical diagnosis of rabies was made and Negri bodies were found in smears made from the brain. On the twenty-third of Sep-

TABLE 1

Data on outbreak of paralytic rabies occurring at miners' camps in British Guiana in 1953

No.	Initials of miners	Camp	Date of arrival	Bat bite	Onset of disease	Duration of disease
1	H.F.	Small's	Not known	Not known	12th Aug	9 days
2	C.L.	Lobert's	Not known	Not known	16th or 17th Aug	2 days
3	D.McP.	Small's	Not known	Not known	1st Sept	6 days
4	C.G.	Furray's	2nd August	Not known	15th Sept	8 days
5	D.D.	Furray's	2nd August	Yes	15th Sept	9 days
6	V.S.	Furray's	2nd August	6 months before	17th Sept	9 days
7	G.A.	Furray's	2nd August	4 weeks before	17th Sept	10 days
8	B.J.	Small's	2nd August	Yes	19th Sept	7 days
9	A.P.	Francis Fevrier's	January	4 weeks before	24th Sept	12 days

tember, another miner was admitted to hospital where he died. He disclosed that two dogs had died in his camp suffering from paralysis of the hind legs, and that he had been bitten by bats. On the twenty-fifth of September two more miners were admitted to hospital who also died.

A visit was paid to the locality and another miner was brought to hospital. He also died. Inquiries revealed that three other miners had suffered from similar symptoms and had died; the first had fallen ill on the twelfth of August, the second on the sixteenth or seventeenth of August, and the third on the first of September. Table 1, shows the day of onset of the disease and other relevant data.

CLINICAL FEATURES OF THE DISEASE

The disease began with tingling and "pins and needles" in the toes and fingers. There was also itching. The sensation passed upward in the limbs and there was pain, which soon passed off. The affected limb became weak and later there was paralysis of the muscles. There was gradual loss of pin-prick sensation commencing distally and proceeding centrally. With paresis of the muscles and impairment of sensation in the legs, the patient had a staggering gait. Later, he was unable to walk. The loss of sensation extended to the upper part of the chest in some instances. There was constipation and inability to pass urine. There was insomnia. The course of the disease was rapid and death occurred in about nine days. Before death, the patient became delirious or spoke idly. In some, there was difficulty in swallowing. Before death, the temperature rose to 103°F.

POSTMORTEM EXAMINATION

Postmortem examinations were made of the bodies of six of the patients dying in hospital. The viscera showed congestion. The bases of the lungs were

edematous. The convolutions of the brain were flattened and there was some edema. The spinal cord was examined in two cases. The meninges were congested. There were no obvious areas of softening.

Smears from the brains of five patients on whom postmortem examinations were made showed Negri bodies. (The brain of the first was not examined by the smear technique for Negri bodies).

CONTROL OF THE DISEASE

As soon as the outbreak was diagnosed, the miners were asked to leave Furray's, Lobert's and Small's Camps. People remained in Francis Fevrier's Camp. Miners were advised to use nets or sacks and to keep a light burning at night. Those bitten by bats were given vaccine against rabies.

DISCUSSION

Acute paralytic rabies in man is a disease requiring further study. It is not known why so few cases have been reported outside of Trinidad. Is the reason the one put forward by Málaga-Alba (1954) who believes that human beings are only slightly susceptible to the disease?

In a locality in which there were forty-three persons, nine suffered from this disease. It is difficult to ascertain the percentage of miners bitten by bats, because they are reluctant to say that they have been bitten. Those bitten probably comprise more than 50 per cent or even 60 per cent of those in the area. Of twenty-three persons in Francis Fevrier's Camp, eighteen, or seventy per cent, said that they had been bitten within the past four months; and four persons, within the past month. There were no cases among people living on the other bank of the creek.

Paralytic rabies was diagnosed in cattle some time before and the occurrence of human cases would indicate the presence of infected animals in the colony or in its neighbourhood and the need for vigilance and for methods of control of the disease.

SUMMARY

The occurrence of the first outbreak of paralytic rabies in man of bat origin in British Guiana is reported. Nine persons were involved in the Mazaruni area, all of whom died in 2 to 12 days after onset.

ACKNOWLEDGEMENTS

I thank Dr. Aurelio Málaga-Alba, Mr. Arthur M. Greenhall and Dr. J. L. Pawan for their advice and assistance; the Director of Medical Services of British Guiana, Dr. L. A. P. Slinger, for permission to publish this report, and Dr. L. J. Charles, Assistant Director of Medical Services (acting), for his help.

REFERENCES

HURST, E. W., AND PAWAN, J. L., 1932. A further account of the Trinidad outbreak of acute rabic myelitis, *J. Path. and Bact.* **35**: 301.

MALAGA-ALBA, A., 1954. Vampire bat as a carrier of rabies, *Am. Jour. Pub. Health.* **44:** 909–918.

PAWAN, J. L., 1936. The transmission of paralytic rabies in Trinidad by the vampire bat, *Ann. Trop. Med.* **30:** 101–130.

PAWAN, J. L., 1936. Rabies in the vampire bat of Trinidad, with special reference to the clinical course and latency of infection, *Ann. Trop. Med.* **30:** 401–422.

PAWAN, J. L., 1939. Paralysis as a clinical manifestation in human rabies, *Ann. Trop. Med.* **33:** 21–29.

PAWAN, J. L., 1948. Fruit-eating bats and paralytic rabies in Trinidad, *Ann. Trop. Med.* **42:** 173–177.

VENTERS, H. D., HOFFERT, R., SCATTERDAY, E., AND HARDY, A. V., 1954. Rabies in bats in Florida, *Am. Jour. Pub. Health* **44:** 182–185.

WITTE, E. J., 1954. Bat rabies in Pennsylvania, *Am. Jour. Pub. Health* **44:** 186–187.

AN ADDITIONAL SPECIES OF INSECTIVOROUS BAT NATURALLY INFECTED WITH RABIES

J. E. GRIMES, R. B. EADS AND J. V. IRONS

State Department of Health, Austin, Texas

The existence of rabies in bats in South and Central America has been known since the early part of this century. Pawan (1936) has recognized the infection in species of fruit eating bats of the genera *Artibeus, Carollia, Dididuras* and *Phyllostoma* in addition to the vampire bats. Johnson (1948) reports that both he and Ten Broeck isolated rabies virus from the vampire bat, *Desmodus rotundus*, in Mexico.

Bat rabies in the United States was first recognized by personnel of the Florida State Department of Health. Venters *et al.* (1954) isolated the rabies virus from a Florida yellow bat, *Dasypterus floridanus*, which was killed while attacking a child. Subsequently, the same investigators, loc. cit., found a second positive species (Seminole bat, *Lasiurus seminolus*) as well as five other infected *D. floridanus*.

Recovery of rabies virus from a third insectivorous species (hoary bat, *L. cinereus*) in Pennsylvania has been reported by Witte (1954). These three species are similar in that they are solitary and their usual daytime retreats are trees and bushes.

Sullivan *et al.* (1954) first isolated the rabies virus from the colonial, cave frequenting Mexican free-tailed bat, *Tadarida mexicana*, from specimens collected in Texas. Central Texas caverns harbor millions of these bats (Plate I), many of which migrate into Mexico during the winter. Consequently, exchange of infection between these insectivores and Mexican vampire bats is an ever present possibility.

The purpose of this paper is to report the recovery of rabies virus from still another species of insectivorous bat, *Chilonycteris personata*, from North America. Two of these bats were collected February 1, 1954, by J. E. Werler, Curator of Reptiles, San Antonio Zoological Society, Texas, from a cave in the Tuxtla Mountains near San Andres Tuxtla, state of Veracruz, Mexico, which was also inhabited by vampire bats.

Both *C. personata* died shortly after being flown to the San Antonio Zoo and one was submitted to this Department for rabies examination. Preliminary determination of the bat (Plate II) was made by Dr. A. Málaga-Alba, Pan American Sanitary Bureau, Mexico City and the specific identity established by Mr. C. C. Sanborn, Curator of Mammals, Chicago Natural History Museum.

The bat was frozen when received in this Department and the brain was unsuitable for direct microscopic examination for Negri bodies. An agent pathogenic for mice was obtained by inoculating four of them with a suspension of brain material. Typical symptoms of rabies were produced and all mouse brains contained Negri bodies. The serum-virus neutralization test was utilized to definitely establish the identity of the agent.

PLATE I

Texas bat cave showing portion of its multimillion *Tadarida mexicana* population.

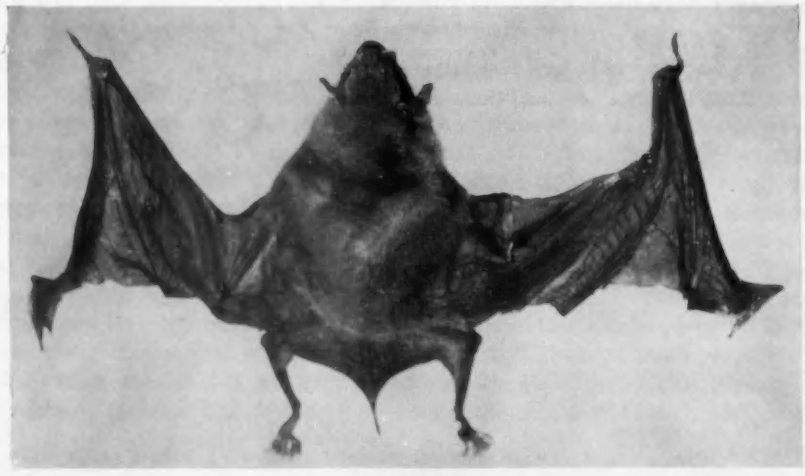


PLATE II

Rabid *Chilonycteris personata* from Veracruz, Mexico

Dalquest and Hall (1949) have collected intensively in the same area in Veracruz in which the rabid *C. personata* was taken. They report that in 1948 this species was encountered in large numbers in a cave near the top of a high cliff. The cave was of two levels, with the lower part inhabited by five species of bats, including *C. rubiginosa*, *Glossophaga soricina*, *Carollia perspicillata*, *Natalus mexicanus* and *Desmodus rotundus*. The upper level contained myriads of *C. personata* and *Pteronotus davyi*, with an occasional *Mormoops megalophylla*. In the cave it was noted that the *Chilonycteris* bore considerable resemblance to small vampires. When handled, they bit viciously, but did not appear to be able to break the skin of a person's hand.

DISCUSSION

This is the second species of North American colonial, insectivorous bat which has been reported to be naturally infected with rabies virus. The *C. personata* from which the isolation was made was collected from a cave in close association with the vampire bat, *D. rotundus*. With the addition of the three positive species of solitary bats, five species of insectivores have been found naturally infected in North America. Interspecies spread of the virus would appear to be a distinct possibility. Transmission such as this might well account for the infected Mexican free-tailed bats, *T. mexicana*, which have been taken in Texas; many of these bats winter in Mexico, within the range of the vampire bat.

REFERENCES

DALQUEST, W. W., AND HALL, E. R., 1949. Five bats new to the known fauna of Mexico, *J. Mamm.* **30:** 424–427.

PAWAN, J. L., 1936. The transmission of paralytic rabies in Trinidad by the vampire bat, *Ann. Trop. Med.* **30:** 101–130.

SULLIVAN, T. D., GRIMES, J. E., EADS, R. B., MENZIES, G. C., AND IRONS, J. V., 1954. Recovery of rabies virus from colonial bats in Texas, *Pub. Health Rep.* (In press.)

VENTERS, H. D., HOFFERT, W. R., SCATTERDAY, J. E., AND HARDY, A. V., 1954. Rabies in bats in Florida, *Am. J. Pub. Health* **44:** 182–185.

WITTE, E. J., 1954. Bat rabies in Pennsylvania, *Am. J. Pub. Health* **44:** 186–187.

A STUDY OF THE OVIPOSITION ACTIVITY OF THREE SPECIES OF *ANOPHELES* IN THE LABORATORY[1]

ROBERT C. WALLIS

The Connecticut Agricultural Experiment Station, New Haven, Connecticut

The problem of selection among oviposition sites by gravid mosquitoes has intrigued entomologists since these insects first became recognized as disease vectors. While field observations strongly supported the premise that restriction of certain species to particular type larval breeding places was a result of selective oviposition activity on the part of the adult female, laboratory experiments designed to demonstrate selectivity among sites have yielded conflicting results. Therefore it was the purpose of this work to find particular factors responsible for confusing results in laboratory experiments with ovipositing *Anopheles*.

Even though salinity as the intrinsic factor differentiating oviposition sites is one of the more clearly defined factors in nature and the one most thoroughly studied in laboratory experiments, such studies have not yielded clear-cut results. Almost thirty years ago Kligler and Theodor (1925) observed that *Anopheles sacharovi*, *Anopheles sergenti* and *Anopheles superpictus* preferred fresh rather than saline water in which to oviposit, although *A. sacharovi* is usually found in nature in brackish water and the other two in fresh water.

DeBuck, Schoute and Schwellengrebel (1932) showed that a brackish water species, *Anopheles atroparvus*, avoided even weak saline solutions and that *Anopheles messeae*, a fresh water breeder, seemed indifferent to saline content of water used for oviposition. Bates (1940) concluded that *A. atroparvus* in Albania was indifferent to sodium chloride in water. He expressed the opinion that sodium chloride may not be the key factor and that some other constituent of sea water such as calcium sulphate might be involved, since he was able to demonstrate a preference for very dilute concentrations of this substance when tested against distilled water. However, Lund (1942) could find no preference on the part of *Anopheles quadrimaculatus* for either calcium or saline solutions. Daggy (1945) showed that *Anopheles farauti* oviposited on all of eleven dilutions of sea water, but that the majority of the eggs were distributed below one and one-half per cent salinity. Causey, Deane and Deane (1943) found that 53 per cent of 15,033 eggs of *Anopheles gambiae* were oviposited on fresh water and 33 per cent on 1 per cent NaCl, and concluded that this species preferred fresh or only slightly brackish water in which to oviposit. Deane, Causey and Deane (1948) gave females of *Anopheles aquasalis* the choice of oviposition on a series of saline solutions of 1, 2, 5, and 10 per cent concentrations. These investigators

[1] This study is part of a research program conducted under the direction of Dr. L. E. Rozeboom, at The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland. It was aided in part by a fellowship from the National Microbiological Institute, the U. S. Public Health Service, Bethesda, Maryland.

found that no eggs were laid on the 10 per cent solution, only a few on the other two, and the majority of the eggs on the 1 per cent solution. De Zulueta (1950) concluded that there was only a slight preference by *Anopheles darlingi* for distilled water over 1 per cent NaCl solution.

Such discrepancies and reports which conflict with findings in the field have been attributed to the fact that oviposition activities of mosquitoes represent highly complex reactions, and the study of such reactions in the very restricted and simplified artificial environment of the laboratory was difficult. In fact Bates (1949) stated, "When selection from a series of oviposition sites can be demonstrated, the basis of the selection may be difficult to demonstrate, and the results may seem unintelligible." However it seemed possible that the process of depositing eggs from flight observed in the oviposition activity of some *Anopheles* (Russell and Rao, 1942; Kennedy, 1942; Bates, 1940) might be responsible for an unnatural distribution of eggs among test sites, and experiments were conducted here to study this activity and its effect on egg distribution among test series.

EXPERIMENTAL PROCEDURE

Tests were undertaken utilizing three species of *Anopheles*. They were *A. quadrimaculatus*, *A. freeborni* and *A. aztecus*. Experiments were carried out in screened cages, 2 x 2 x 2.5 feet in size, at room temperature of 76 ± 4°F, in which adult populations of over 200 mosquitoes were maintained. The first approach consisted of close observation of individuals of each species during many periods of oviposition activity to determine the exact activity pattern when a series of test solutions was introduced into the cage. The test series consisted of seven 65 sq. cm. Petri dishes placed 5 cm. apart. One dish contained 60 ml. of distilled water and the other six contained 60 ml. each of solutions ranging from 1 to 6 per cent NaCl. Since the height of oviposition activity in the colonies was in the early mornings and late afternoons when daylight was subdued, observations were facilitated by using monochromatic red light (which is beyond the wavelength of visibility to the mosquito) to illuminate the interior of the cage with a minimum of disturbance to the mosquitoes.

To test the extent of overlapping effect, an experiment was set up in which a dish containing distilled water was placed in the cage of *A. quadrimaculatus*. Around the sides of the water dish, four dry Petri dishes were placed at the same distance as the saline dishes had been in the first test series—approximately five cm. apart—at a time when oviposition activity was high. Mosquitoes that began oviposition while resting on the water were disturbed with a long, stiff wire introduced through the screen of the cage so as to prevent contact oviposition and insure that all eggs were dropped from flight. In this fashion an experimental period of an hour was maintained. Then the dishes were removed and the eggs counted to obtain an approximation of the percentage of eggs that overlapped onto adjacent dishes. The same procedure was used with the *A. aztecus* and *A. freeborni* colonies. Since it was noted that the flight patterns for the three species differed considerably and wider separation of dishes might

minimize the overlapping, the same procedure was repeated with the dry dishes separated from the distilled water dish by 10 cm.

After the approximation of the percentages of eggs that overlapped was obtained, it was necessary to ascertain what proportion of the total number of eggs had been oviposited from flight. An experiment was devised to do this in the following manner: for each test two large Petri dishes 15 cm. in diameter were set up. Over each, on a platform of two thin wooden rods, a 43 x 50 mm. glass cover slip was mounted just above the surface of the water. Each Petri dish provided 176.6 sq. cm. and its cover slip 21.5 sq. cm. of surface area. When 200 ml. of distilled water was poured into the dish, 155.5 sq. cm. of water surface was exposed, representing approximately 7 times the area of the cover slip. Both dishes, one dry and one containing water, were placed in the mosquito colonies so that a distance of 10 cm. separated the edges of the cover slips over the wet and dry dishes. This procedure was followed for each of the three *Anopheles* colonies during a twenty-four hour period and the eggs on the respective surfaces counted and recorded. In this way an index of the percentage of total eggs deposited from flight as compared to those deposited by contact was determined, as well as a recheck of the amount of overlapping that took place in the processes.

RESULTS

In the first series of observations mosquitoes were seen ovipositing both while in contact with the water surface and while flying over the distilled water dish. It was quite evident that eggs dropped from this oviposition flight were scattered beyond the restricted distilled water area and landed on solutions in the test series that were adjacent to the distilled water dish. When the positions of the dishes in the series were changed around to avoid a "position preference", this scattering of eggs gave the same effect as an indiscriminate distribution

TABLE 1

Estimation of the number of eggs that over-lapped into dry dishes placed at a distance of five and ten centimeters from a distilled water site

Species	Total no. eggs on dist. water	Dry dishes, no. eggs on each dish	M*	Ratio of Dry/100 wet
Dishes separated by five centimeters				
A. quadrimaculatus	334	65 121 88 202	119	35.6
A. freeborni	214	10 18 7 12	11.8	5.5
A. aztecus	19	2 0 1 1	1	5.2
Dishes separated by ten centimeters				
A. quadrimaculatus	273	2 8 9 6	6.2	2.3
A. freeborni	302	7 0 10 14	7.7	2.5
A. aztecus	24	3 1 0 2	1.5	6.3

* Mean number of eggs per dry dish.

such as would result from oviposition without selection among the sites in the test series.

The results recorded in Table 1 give an index of the amount of over-lapping of eggs oviposited during flight by these three species and are quantitative evidence of the different flight patterns observed. It can be seen that in the cases of *A. quadrimaculatus* and *A. freeborni*, whose flight patterns while laying eggs were somewhat localized over the water area, the simple procedure of moving the dishes from 5 to 10 cm. apart effectively reduced the amount of overlapping. However, in the case of *A. aztecus*, whose flight pattern was such that the mosquito ranged over considerable areas, the separation of dishes by as much as ten centimeters did not reduce the percentage of overlap onto adjacent dishes.

The procedure described for the next experiment was designed to obtain an approximation of the proportion of eggs oviposited from flight and the results are given in Table 2. Here the number of eggs on the collecting plate (Y) represented the number of eggs deposited from flight for that surface area. This number was multiplied by 7.21 to determine how many eggs were deposited from flight over the exposed water area. The number (Y × 7.21) was subtracted from the total number of eggs counted on the water surface (X), and the resulting number (X-(Y × 7.21)) represents the number of eggs deposited by mosquitoes while resting on the water surface. (X) and (Y) were then added to give the total number of eggs deposited. From this, percentages were calculated representing indexes of the proportions of eggs oviposited while in contact, from flight, and percentages of eggs overlapping onto adjacent dishes at approximately 10 cm. (10 ± 2.5 cm.). Thus the results in Table 2 indicate that under the conditions of these tests 88 per cent of eggs of *A. quadrimaculatus* were from flight. This is compared to 8 per cent for *A. freeborni* and 1 per cent for *A. aztecus*.

TABLE 2

Eggs deposited from flight by Anopheles *on collecting plates, on water surfaces, and on adjacent test areas*

	A. quadrimaculatus		*A. freeborni*		*A. aztecus*	
	No. eggs on water dish	No. eggs on dry dish	No. eggs on water dish	No. eggs on dry dish	No. eggs on water dish	No. eggs on dry dish
No. eggs on collecting plate (Y).....	285	29	18	14	2	1
Estimated no. from flight (Y × 7.21)..	2054	216	129	101	14.4	7.2
Total no. eggs on the water surface (X).....	2372	209	1645	388	1444	180
Estimated no. from contact X − (Y × 7.21).....	318	0	1516	0	1430.4	0
Total no. eggs (X + Y).....	2657	238	1663	402	1448	182
Estimated % eggs by contact.....	11.96		91.17		98.89	
Estimated % eggs from flight.....	88.04		8.83		1.11	
Estimated % eggs overlap at 10 cm...		8.9		24.1		12.5

Of these 8 per cent for *A. quadrimaculatus*, 24 per cent for *A. freeborni*, and 12 per cent for *A. aztecus* overlapped into adjacent dishes.

DISCUSSION

In past experiments with *Anopheles*, in which attempts were made to demonstrate a preference on the part of the ovipositing mosquito among a series of test sites differentiated only by saline content of the water, the procedure has been one of introducing a series of test solutions in containers such as dishes, bowls, pans, etc. into the cage. These were routinely shifted around in order to avoid any possibility of a "position preference" for any one location influencing egg distribution into any particular dish. Such a simple procedure resulted in clear-cut results with species of *Aedes* (Woodhill, 1941) and *Culex* (De Zulueta, 1950). However, in testing *Anopheles* mosquitoes it can be seen that such a shifting around of the dishes only serves to give each an equal chance of catching eggs oviposited from flight which accidentally overlap from the preferred sites onto solutions not selected. The amount of this overlapping into repellent solutions depends upon several factors. The species of *Anopheles* used in the test caused the amount to vary because of differences in flight patterns exhibited by various species (Wallis, 1954). Mosquitoes which have an activity pattern confined to predominantly up-and-down movements over a restricted area have less overlapping than those which range over a considerable area in their oviposition flight. In the case of the former, relatively small increases in distance between test sites greatly reduced the per cent of overlapping and resulted in more clear-cut demonstrations of preferences between sites. Without this special consideration in experimental procedure there was generally a sufficiently high percentage of eggs deposited on distilled water to justify the conclusion that fresh water was preferred, although approximately 30 per cent of the eggs were scattered across solutions ranging up to 5 per cent. This is well over the 3 per cent concentration of salinity in sea water and these results have been interpreted by previous investigations as lack of selectivity.

In the literature the real inconsistency in laboratory findings was centered around the three fresh water species for which selection of fresh water had not been shown in laboratory experiments. One of these was Lund's (1942) conclusions from work with *A. quadrimaculatus*, in which no statistical difference could be demonstrated regarding selection among saline sites. However, inspection of Lund's methods show that he placed twelve dishes of solutions in a cage comparable in size to those used in this study. Measurements of the cage indicate that insufficient space was provided for separation of the dishes adequately to prevent overlapping. The other two cases of conflicting reports, De Zulueta (1950) and De Buck *et al.* (1932) may be accounted for on the same basis as Lund's.

While an indication of the amount of overlapping of egg distribution among test sites was extremely useful in demonstrating more clear-cut preferences among test solutions in experiments, caution must be used in evaluating results

of other experiments because of the variability of this factor. The amount of overlapping of distributions is directly related to the proportion of the total number of eggs oviposited from flight. This in turn varies from time to time within an *Anopheles* colony according to conditions. Bates (1940) suggested it is probably related to cage size and adult populations present. He estimated the proportion of eggs oviposited from flight by making counts of eggs scattered at random over the water surface, and compared them with counts of eggs gathered in "batches" on the surface of the water in order to estimate the proportion of eggs oviposited from flight. Observations here indicate that such a procedure may not be justified, as much clumping of eggs was observed due simply to surface action forces of the water, rather than to the method of oviposition. Since the individual flight patterns utilized by different species while ovipositing from flight affect the amount of overlapping in test series, the wet-dry plate method used here for actually determining the effect of this factor on egg distribution among test sites at the time when selection tests are undertaken is recommended since direct observations of flight patterns are difficult in the fast-moving activity of an *Anopheles* colony. For this reason, the wet-dry plate method of determination is superior in that it gives a quantitative measurement more useful in determining amounts distributed among saline oviposition sites. The flight pattern of *A. quadrimaculatus* over a test site was confined to a very restricted area, so that the separation of dishes in a test series by only an additional five cm. resulted in greatly reducing the amount of accidental overlapping of eggs among collection sites. The flight pattern of *A. freeborni* was not so restricted over the test site, and separation of dishes in test series by an extra five cm. resulted in little reduction in the amount of overlapping. The distribution of eggs of *A. aztecus*, whose flight pattern was unrestricted and far-ranging, was unaffected by an extra five cm. separation of dishes. Since the amount of overlapping depended upon the proportion of the total eggs oviposited from flight, procedures for estimation of the proportion of eggs dropped from flight by collection on dry surface sample plates were described. Under the conditions of these tests *A. quadrimaculatus* oviposited from flight approximately 88 per cent of the total eggs collected. This was considerably different from the 8 per cent for *A. freeborni* and 1 per cent for *A. aztecus*. The distribution of these eggs from flight was considered the chief source of error in oviposition selection experiments.

SUMMARY AND CONCLUSIONS

Tests were designed to determine the effect of oviposition from flight of three species of *Anopheles* in laboratory work concerning selection among oviposition sites. Eggs oviposited from flight were scattered over wider areas than test sites, and the accidental overlapping distributions of eggs gave the effect of apparent indiscriminate distribution and lack of selection.

REFERENCES

BATES, M., 1940. Oviposition experiments with *Anopheline* mosquitoes, *Amer. Jour. Trop. Med.* **20**: 569–583.

——, 1949. *The Natural History of Mosquitoes*. New York, Macmillan, pp. xv-379.

CAUSEY, O. R., DEANE, L. M., AND DEANE, M. P., 1943. Ecology of *Anopheles gambiae* in Brazil, *Amer. Jour. Trop. Med.* **23**: 73–94.

DAGGY, R. H., 1945. The biology and seasonal cycle of *Anopheles farauti* on Espiritu Santo, New Hebrides, *Ann. Entom. Soc. Amer.* **38**: 1–13.

DEANS, L. M., CAUSEY, O. R., AND DEANE, M. P., 1948. Notas sôbre a distribuição e a biologia dos *Anofelinos* das regiães Nordesina e Amazonica do Brazil, *Rev. do Serviço Especial de Saude Publica*. **1**: 827–965.

DE BUCK, A., SCHOUTE, E., AND SWELLENGREBEL, N. H., 1932. Further investigations on the racial differentiation of *Anopheles maculipennis* in the Netherlands and its bearing on Malaria, *Riv. Malariol.* **11**: 137–156.

DE ZULUETA, J., 1950. Comparative oviposition experiments with caged mosquitoes, *Amer. Jour. Hyg.* **52**: 133–142.

KENNEDY, J. S., 1942. On water finding and oviposition by captive mosquitoes, *Bull. Entom. Res.* **32**: 279–301.

KLIGLER, I. J., AND THEODOR, O., 1925. Effect of salt concentration and reaction on the development of *Anopheles* larvae, *Bull. Entom. Res.* **16**: 45–49.

LUND, H. O., 1942. Studies on the choice of a medium for oviposition by *Anopheles quadrimaculatus* Say, *Jour. Nat. Mal. Soc.* **1**: 101–111.

RUSSELL, P. F., AND RAO, T. R., 1942. On the swarming, mating and oviposition behavior of *Anopheles culicifacies*, *Amer. Jour. Trop. Med.* **22**: 417–427.

WALLIS, R. C., 1954. A study of the oviposition activity of mosquitoes, *Amer. Jour. Hyg.* **60**: 135–168.

WOODHILL, A. R., 1941. The oviposition responses of three species of mosquitoes (*Aedes* (*Stegomyia*) *aegypti*, *Culex* (*Culex*) *fatigans*, *Aedes* (*Pseudoskusea*) *concolor*) in relation to the salinity of the water, *Proc. Linn. Soc. New South Wales* **66**: 287–292.

BOOKS RECEIVED

Kwashiorkor, by H. C. Trowell. 308 pp., illustrated. Baltimore: Williams & Wilkins Company. 1955. $10.00.

La Toxoplasmosi, by Paolo Tolentino and Antonio Bucalossi. Rome: Il Pensiero Scientifico. 1955.

American Standard National Plumbing Code, by American Society of Mechanical Engineers, 174 pp., illustrated. New York: American Society of Mechanical Engineers. 1955.

Reactions with Drug Therapy, by Harry L. Alexander. 301 pp., illustrated. Philadelphia, London: W. B. Saunders Co. 1955.

Biochemistry and Physiology of Protozoa, Vol. II, by Hutner and Lwoff (Eds.). 388 pp., illustrated. New York: Academic Press, Inc. 1955. Price $9.00.

Poliomyelitis, WHO Monograph Series No. 26, by Robert Debre and 16 other contributors. 408 pp., illustrated. Geneva: Palais des Nations, WHO, distributed in the U.S. by International Documents Service, Columbia University Press, 2960 Broadway, New York 27, N. Y. 1955. Price $8.00, £2, Sw.fr. 24.

Ion Exchange and Adsorption Agents in Medicine—The Concept of Intestinal Bionomics, by Gustav J. Martin. 333 pages, illustrated. Boston, Toronto: Little, Brown & Co. 1955. Price $7.50.

Antisera, Toxoids, Vaccines and Tuberculins in Prophylaxis and Treatment, by H. J. Parish. 3rd edition. 227 pp., illustrated with 19 in color. Baltimore, Williams & Wilkins Co. for the Wellcome Foundation Ltd. 1954. Price $5.00.

Manual of Medical Helminthology, by C. J. Hackett with the cooperation of Professor J. J. C. Buckley and the late Professor F. Murgatroyd. 330 pp., illustrated. London: Cassell and Co., Ltd. 1954. Price 18/6 net.

Foetal, Infant and Early Childhood Mortality: Volume I—The Statistics, Volume II—Biological, Social and Economic Factors. Population Studies No. 13, by United Nations, Dept. of Social Affairs, Population Divn. Volume I—137 pp., Volume II—43 pp. United Nations through International Documents Service, Columbia University Press, 2960 Broadway, New York, N. Y. Price Volume I—$1.50, Volume II—.40.

Expert Committee on Drugs Liable to Produce Addiction, Fifth Report (WHO Technical Report Series No. 195). 16 pp. Geneva, Palais des Nations: WHO distributed in the U.S. through International Documents Service, Columbia University Press, 2960 Broadway, New York, N. Y. Price 1/9, $0.30, Sw.fr. 1.

Practical Medical Mycology, Pub. Number 248 American Lecture Series, by E. L. Keeney, edited by R. L. Pullen. 145 pp., illustrated. Springfield, Ill.: Charles C Thomas Co. 1955. Price $3.75, $4.50.

BOOK REVIEWS

Histology, edited by Roy O. Greep, Ph.D., Dean and Professor of Dental Science, Harvard School of Dental Medicine, with thirteen contributors. 953 pp., 648 illustrations, with many in color. New York & Toronto: The Blakiston Co., Inc., 1954. Price $15.00.

Professor Greep's compendious Histology is the most recently published textbook in English on the subject, and in it one would therefore expect to find new concepts of the finer structure of the body. In this the reader will not be disappointed. The illustrative treatment exemplifies the editor's aim to present the subject with the use of "new tools and new techniques, applied either to living or fixed tissues." In the employment of newer methods, the work is notable among the six or seven current American texts in its subject, and can be compared favorably with the two or three notable treatises—those of Ham and Maximov-Bloom.

Conventional pictures secured from ordinary sections and stained with ordinary hematoxylin and eosin, which are wearisome in the extreme, are almost absent. There are perhaps 20 of them in the entire book; but there is a flood of new images from electron microscopy and phase contrast as well as from the polarization microscope and from radio-autographs. If a carping critic becomes tired of the application of a few histochemical procedures, for example the periodic acid-Schiff reagent, Bodian silver and Gomori, he is not thereby entitled to stigmatize the book; and the brief but excellent chapter on histochemistry is a welcome innovation.

The wealth of fresh and significant illustrations is exemplified in the treatment of reproductive physiology: witness the Corner figures on the development of the corpus luteum of macacus, and Hertig and Rock figures of the human ovum and blastulae, and the astonishing little picture which depicts what Sudan black can do with human spermatozoa where the spiral filament of the middle piece is selectively portrayed.

As is inevitable, the chapters are of uneven worth. The information and judgment used in the brief introductory chapter by Dempsey is praiseworthy. Such texts do not usually treat either the sense organs or the teeth, except cursorily, but tooth structure has certainly received a better treatment here than is usually the case; and the chapter on placentation, expectedly excellent from its authorship, is a notable one and contains new and unpublished material. When accounts are so excellent as that on the mesenchyma and connective tissues, it is all the more regrettable that a better account of osteogenesis is not given us or that the endocrine factors in bone formation are restricted to those exercised by the gonads. Perhaps some of the inadequate descriptions and illustrations, such as those of the carotid body, the Keith-Flack and Tawara nodes and of the Purkinje system, reflect actual paucity of our knowledge. On the other hand the judgment shown in the editor's own brief chapters on the adrenal and pituitary will win general acclaim.

Over and above all, probably the outstandingly good characteristic of the book consists in its frequent discussions of the experimental attack: witness the discussion of the participation of the blood cells in such fields as antibody production, allergy, and the Rh factor. The kind of evidence which permits us to make a good approximation of the length of the life span of erythrocytes, even though such a discussion is not complete, is a good case in point and the situation regarding the life span of the white corpuscles is critically discussed. No more excellent example could be furnished than the treatment of the lymphatic system with proper employment of the beautiful monograph by Drinker and Yoffe.

The Greep treatise is, fortunately, not intended to drill elementary students in conventional facts. It is written from physiological standpoints with thought, which it presupposes in its student readers. It conducts them at once to the experimental aspects of the subject and can hence play a good role in the best type of medical education.

HERBERT M. EVANS

Plague, by R. Pollitzer M.D. (formerly of the Division of Epidemiological and Health Statistical Services, WHO), 698 pp., illustrated, with 2 in color. WHO Monograph Series No. 22. New York, Columbia University Press, International Documents Service, 1954. Price $10 cloth, $9 paper; £ 3/5/0 cloth, £ 3/0/0 paper; Sw. fr. 40 cloth, Sw. fr. 36 paper; (French edition in preparation).

The publication of this critical and definitive volume on the biology of plague is a most important event in itself in the history of knowledge on the subject. It is indeed a worthy summing up of all aspects of the host-parasite relationship in plague and is a masterful example of a form of scholarship which has all but disappeared from the literature of medical microbiology and infectious diseases.

The author has arranged the presentation in ten chapters, among which to the reviewer the most outstanding are those devoted to the history and distribution of plague, the epidemiology and the control and prevention of plague. In the historical section some controversial problems are thoroughly examined. The weighing of evidence in this and indeed in all subsequent chapters is prodigious.

The chapters dealing with bacteriological and immunological problems are exceedingly

useful in their content of critical discussion. The author did not appraise the usefulness of the quantitative immunochemical approach in preparing or assaying the various antigenic fractions, certain of which are active immunizing fractions and this to the reviewer is the only major omission in the book. On the more positive side Dr. Pollitzer clearly shows the frustration to which a consideration of the present state of the taxonomy of *Pasteurella pestis* must lead. Certainly the Parvobacteriaceae appear to contain a strange collection of microorganisms, hardly one genus of which is a satisfactory taxonomic entity.

The chapters dealing with hosts of the infection and with insect vectors, together with the appendices to the book covering identification of fleas and lists of reservoirs, are sufficient to stand by themselves as compendia.

The chapter on pathology is a most distinguished one for its beautiful colored plates and the detailed review of the appearance of the infection in all important hosts. The author's position on the pathogenesis of pneumonic plague carries a clear and concise statement regarding primary versus secondary pulmonary plague. It is accompanied by a critical consideration of the kinds of data necessary for the interpretation of results from intranasal, intratracheal and inhalatory routes of exposure. To the experimental pathologist, rare breed today, this chapter offers much to whet the curiosity concerning the ultimate explanation of the pathogenesis of plague.

A chapter on clinical aspects is a complete statement of the disease as a clinical entity. Sixty-seven pages here are devoted to every aspect of the disease itself and its therapy.

Throughout, the book is also a gold mine of techniques and data; these are presented with much discrimination. It is therefore not too much to claim that the book will be as useful on the laboratory bench as it is in the field pack of the biological survey and rodent control group, on the desks of the entomologist as well as of those in preventive medicine and public health!

Dr. Pollitzer and the World Health Organization have published a book of encyclopaedic proportion which will serve as a model for monographs on specific infectious diseases. The extent of the bibliography is enormous, each chapter having from 100 to 200 well-selected papers upon which the discussions are based. Throughout the book, the author's own lifetime of study on all aspects of this specific parasitism serves as a constant source of personal experience so that the book is truly a living work which tells of the past, present and future of Plague.

SANFORD S. ELBERG

Medical Mycology, edited by R. D. G. Ph. Simons, Senior Lecturer at the Dermatological Clinic of the University of Leyden, Dermatologist in Charge at the Civilian Hospital, Amsterdam; with 36 contributors. 446 pp., illustrated. Amsterdam, Houston, New York, London: Elsevier Press, 1954. Price $9.75.

This book, as stated in the preface, represents the section on mycology of Volume II of the Handbook of Tropical Dermatology and Medical Mycology edited by Simons. It is concerned not only with the tropical but also the nontropical mycoses. There are additional sections by Emmons on the development of medical mycology which is well presented, and a series of photographs of cultures with one plate of 15 cultures in color by Ajello.

The list of 36 contributors is made up of some that are well known and others not well established as medical mycologists. Each has contributed one and in some cases more sections to the book, which is an attempt to cover all the known mycoses and various phases of the subject such as physiology, techniques, experimental infections in laboratory animals and therapy. These features and in addition the cultural characteristics of pathogenic fungi and of the saprophytes commonly seen in the laboratory are concise and well written although in some instances somewhat brief.

There are several criticisms of the book which detract greatly from its value. The outstanding discrepancy appears to be in the conflict of terminology. The names of the organisms have not been standardized throughout the book. The presentation of the mycoses in a topographical fashion results in needless repetition of subject matter and reduplication of

photographic material in some cases. Although there is an abundance of clinical photographs, some almost repetitious, there are too few photographs of the histopathologic features. Errors in spelling are found throughout.

The section on moniliasis by Benedek is a general nonacceptance of the pathogenic role of *Candida albicans* in superficial skin lesions. He terms this saprophytosis. This plus his denial of the etiologic role of *Candida albicans* in proven cases of granulomatous moniliasis detract greatly from his presentation. His concept of saprophytosis is further applied to the onychomycoses where he states that the underlying cause is pompholyx and not a direct result or manifestation of fungus pathogenicity. On page 217 he makes the sweeping statement which many will undoubtedly oppose that "One fact stands out among the various claims for the pathogenicity of known pathogens, saprophytes, hyphomycetes and yeast-like organisms in the clinical picture called "onychomycosis". This is the identical clinical picture of the nail organ in the pathological process."

Throughout the book there are italicized statements presumably to emphasize the point in question. Some of these are clearly disputable such as that made by Ajello on page 71. "The finding of mycelial filaments in skin scrapings indicates the mycotic nature of the infection but does not reveal the identity of the etiological agent, since all members of the genus *Trichophyton* produce identical structures in the skin." Actually, a careful observer should be able to differentiate many of the dermatophytes including the trichophytons even so far, in some cases, as species. On page 87 Marples states that "the appearance of *M. gypseum* under Wood light and in skin scrapings is indistinguishable from that of *M. canis*". This is not true since *M. gypseum* forms larger spores and thicker hyphae than do *M. canis* and *M. audouini*.

Throughout the book there are statements concerning the taxonomy of the fungi with which this reviewer does not agree. There are, however, some redeeming features such as the concise clinical descriptions of the systemic diseases and of some of the superficial mycoses. These, however, do not completely offset much of the criticism made above.

MORRIS MOORE

Malnutrition in African Mothers, Infants and Young Children, Report of the Second Inter-African Conference on Nutrition held under the auspices of the Commission for Technical Co-operation in Africa South of the Sahara (C.C.T.A.), 398 pages, illustrated. London: Her Majesty's Stationery Office, 1954. Price £ 1.5s.

As indicated by the title, this volume includes summaries of the papers and discussions of the Second Inter-African Conference on Nutrition held in Gambia in 1952. Most of the papers are related to the disease or syndrome usually called kwashiorkor in infants and young children. The scope of the conference is indicated by the titles of the sections on the conference proceedings. These include Clinical Features, Demography, and Nosography; Pathology; Biochemistry; Dietetics; Malnutrition in Mothers and Treatment and Prevention of Malnutrition in Infants and Children; Terminology; and Recommendations. The proceedings thus supply an excellent summary of present knowledge by many of the pioneers in the field and others best qualified to evaluate the evidence.

In view of the numerous papers and topics it is not possible to summarize the contents of the volume. It is apparent that one of the serious questions facing workers in this field is whether or not kwashiorkor is a disease entity or a number of syndromes with some common etiological factors. Undoubtedly most of the participants at the congress believe that protein deficiency is the principal underlying cause. In view of the very limited knowledge of the protein and amino acid requirements of the child at this age, the evidence is only indirect. The final and classical proof as to the cause of a nutritional disease must be response to specific therapy. Strangely enough there seems to have been rather few attempts to treat kwashiorkor with highly purified proteins or amino acid mixtures and these do not seem to have offered much promise. Treatment with skim milk powder, the preferred method, can scarcely be considered specific protein therapy. The curious or significant parallel between pellagra and kwashiorkor recalls that there was, and still is,

considerable reason to consider pellagra a protein deficiency. The reviewer notes only one author at the present conference who states his belief that pellagra and kwashiorkor are essentially the same disease.

The disease kwashiorkor or its variants is an extremely widespread and serious nutritional disease and it is obviously important to bring together widely separated workers from throughout the world. The conference proceedings also succeed very well in supplying an extensive bibliography from all parts of the medical literature. The many groups now working upon the various aspects of kwashiorkor offer promise that the numerous perplexing problems related to the causative agent or agents, biochemical defects, variations in symptomology, etc., will soon be solved. Even at this stage the therapeutic and preventative measures can be reasonably well defined and emphasize the magnitude of the problem. Social, educational, economic, and agricultural aspects are as important as the purely medical aspects and, as this conference concludes, the cooperation of practically all the technical, social, and administrative departments of the government and voluntary agencies will be required for the ultimate solution.

D. MARK HEGSTED

Diseases of the Skin for Practitioners and Students, by George Clinton Andrews, M.D., F.A.C.P. Clinical Professor of Dermatology, College of Physicians and Surgeons, Columbia University; Attending Dermatologist to the Presbyterian Hospital, Columbia-Presbyterian Medical Center, New York. 4th edition: 877 pp., 77 illustrations. Philadelphia and London: W. B. Saunders Co., 1954. Price $13.00.

The fourth edition of this well-known text has been improved and pulled together. There are added numerous good sections—for example on such fundamental matters as the newer concepts of melanogenesis, pigmented nevi and tumors; on the "lupus erythematosus phenomenon" and the Kveim test in diagnosis; on Thorium X, atabrine and chloroquine in treatment.

The vastness, complexity and diversity of the arts and sciences which must be included and described in a modern "Compleat Dermatology" suggest that the heyday of a "one-man text" may be waning. No one person can hope to be equally proficient in such widely differing fields as dermatologic biochemistry and histochemistry; dermatologic microbiology and immunology; and the clinical diagnosis as well as the specialized surgery, radiation, physical therapy and external topical treatment of the hundreds of different skin diseases, to name but a few examples.

It is therefore almost inevitable that such an encyclopedic text as this, to all practical purposes the work of one man, should evidence considerable inequalities. In this reviewer's opinion the sections on Eczema—Contact Dermatitis—Atopic Dermatitis, for example, fall somewhat short of the general excellence of such chapters as those on Radiation and Radiation Therapy, not to mention the sensitive beauty of the dedication to the memory of Dr. Richard B. Coutant.

The therapeutic directives, are modernized and generally adequate. In a few places they are not quite detailed enough to satisfy this reviewer's concept of what will prove of actual practical use to the nonspecialist or even the beginning specialist.

Despite such minor imperfections, Dr. Andrews' long personal experience, coupled with his excellent compilations and integration of the experience of others, have produced a book which is admirably complete, clear and concise. It is predictable that this edition will more than hold its own among its rivals unless and until a new text appears which is the work of many authors, each contributing sections on just those selected aspects of the specialty on which he is particularly and preeminently qualified to write.

MARION B. SULZBERGER

Histopathologic Technic and Practical Histochemistry, by R. D. Lillie, A.B., M.D. Medical Director, U. S. Public Health Service; Chief, Pathologic Anatomy Service, Clinical Center, National Institutes of Health; and Chief, Laboratory of Pathology and Pharma-

cology, National Institute for Arthritis and Metabolic Diseases. 456 pp. New York and Toronto: The Blakiston Company, Inc. 1954. Price $7.50.

This book is probably the most complete available account of techniques required for the demonstration of the structural and chemical properties of cells and tissues localizable by means of the ordinary light microscope. It treats lightly or leaves out details concerning the use of complicated equipment, such as the finer grades of ultracentrifuges, spectrographs, polariscopes and electron microscopes.

The early chapters describing microscopical equipment, fixation, staining and so on are clearly presented and suitable for beginners. In the case of more difficult techniques precision is given by specifying the source of the dyes and other reagents employed. A few well chosen references to the literature are supplied and helpful tables giving the composition of buffers and normal acids and alkalis. The author, a master of histopathology, gives valuable help by reference to his personal experience in the use of many important methods. Of particular value are the chapters on the microscopic localization of enzyme activities, pigments and unicellular organisms such as bacteria and *Rickettsia*.

E. V. COWDRY

Wine as Food and Medicine, by Salvatore P. Lucia, M.D., Sc.D., 149 pp., New York and Toronto, The Blakiston Company, Inc., 1954, $3.00.

In the preface to this book the author states that he has approached his subject with "great warmth and fondness." His enthusiasm is readily apparent to the reader and at times there must be question whether objectivity and scientific accuracy has not been compromised in some measure as tribute is paid to an important and much enjoyed beverage.

The first chapter deals with the chemistry of wine and the process of fermentation. It is both informative and interesting to read. In consideration of the contributions which wine may make as a food, data are recorded giving the calories afforded per unit measure of a variety of wines. Wine is also proposed as a food providing essential mineral elements, of which iron is present in most significant quantity. The author contends that wine may contribute importantly in meeting the daily needs for vitamins of the B complex but no data are given to substantiate this statement. Actually, with moderate intake it is unlikely that this end would be achieved.

The physiologic effects of wine are reviewed and appraisal is made of the opportunities for the use of this beverage in therapy. Undoubtedly, wine could be used more frequently and to good advantage by the physician in prescribing for the needs of his patients. This point is made even more strongly by the author when he states "the generalized vasodilatation, the increased flow of blood to the skin and the mild euphoria wine produces are as necessary as they are gratifying to both the aged and the convalescent."

The book represents a useful compendium on wine. It is readily readable with a literary style somewhat more flamboyant than is usual in scientific or medical literature. There are more than three hundred references in the bibliography. Of this number nearly one-half are to publications which appeared before 1930 and indeed one of the most attractive things that the reader will find in the book is the historic detail with which it is embellished.

ROBERT E. SHANK

Care of Laboratory Animals, By Subcommittee on Diagnostic Procedures and Reagents and Approved for Publication by the Coordinating Committee on Laboratory Methods and by the Committee on Research and Standards of the American Public Health Association, Inc. 66 pages, illustrated. New York: American Public Health Association, 1954. Price, 1–19 copies—75¢; 20–99 copies—65¢; 100 copies and over—50¢. Prepared by C. A. Slanetz, Ph.D.

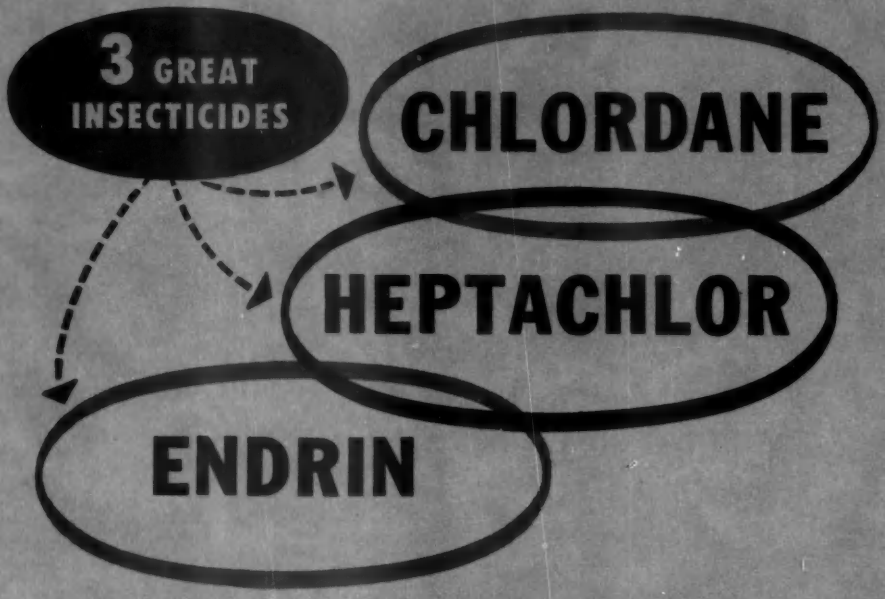
This pamphlet has been prepared for animal caretakers in biological and medical institutions. Basic information is summarized in simplified outline form regarding the daily care and maintenance of the common laboratory animal species. For example, fundamental

routines are described for housing, feeding, and watering laboratory animals, and for maintaining adequate standards of sanitation and disease control in animal quarters. Minimal requirements are suggested for cage space per animal, temperature and humidity controls, and food selection and storage.

The limited objectives of the pamphlet are emphasized by Doctor C. A. Slanetz: "The handbook is not intended to be an exhaustive discussion of any phase of animal care." There are several omissions, however, which might properly have been included in describing a caretaker's work routine, which would have added to the value of this publication. Thus, except for the cotton rat, no mention is made of simple techniques for handling commonly used species. Further, the essential role of the animal caretaker in maintaining a stable environment for laboratory animals, while implied, is not directly stated, as it should be in an elementary presentation. As an additional criticism, the animal cage photographs did not reproduce well, showing very little detail. It is questionable too, whether the best of the commercially available "typical" cages are pictured.

Despite these shortcomings, this is a useful publication, since it provides a concise, written framework around which sound daily work routines in animal quarters can be developed. The pamphlet should be made available to animal caretakers to complement their practical training and as a handy reference on basic laboratory animal care.

BENNETT J. COHEN



CONTROL THESE INSECTS

CHLORDANE: Ants, Armyworm, Blister Beetles, Boxelder Bug, Brown Dog Tick, Cabbage Maggot, Carpet Beetles, Cattle Lice, Chiggers, Chinch Bug, Cockroaches, Crickets, Cutworms, Darkling Beetles, Dog Mange, Earwigs, Fleas, Flies, Grasshoppers, Household Spiders, Japanese Beetle Larvae, Lawn Moths, Lygus Bugs, Mole Crickets, Mosquitoes, Onion Maggot, Onion Thrip, Plum Curculio, Sarcoptic Mange, Seed Corn Maggot, Sheep Ked, Silverfish, Sod Webworms, Southern Corn Rootworm, Strawberry Crown Borer, Strawberry Weevils, Sweet Clover Weevil, Tarnished Plant Bug, Termites, Ticks, Wasps, White Grubs, Wireworms . . . and many others.

HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Black Vine Weevil, Cabbage Maggot, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevils, Cotton Thrips, Cowpea Curculio, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrip, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Serpentine Leaf Miner, Spittle Bug, Strawberry Root Weevil, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Turnip Maggot, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms . . . and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevils, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

WRITE FOR FULL PARTICULARS

VELSICOL CORPORATION

Division of Arvey Corporation

General Offices and Laboratories
330 East Grand Avenue, Chicago 11, Illinois

Export Division
100 East 42nd Street, New York 17, New York

REPRESENTATIVES IN PRINCIPAL CITIES

PETROLEUM DERIVED CHEMICALS
VELSICOL
SERVING BASIC INDUSTRIES

DIFCO

BRUCELLA

Isolation, Cultivation and Differentiation

BACTO-TRYPTOSE

is the peptone of choice in the preparation of both liquid and solid media for culturing *Brucella abortus, melitensis* and *suis* and supplies the nutriments required by these organisms for rapid and abundant growth.

BACTO-TRYPTOSE BROTH

is a complete liquid medium for culturing the *Brucella* and is especially adapted to the isolation techniques recommended by Huddleson and Castaneda.

BACTO-TRYPTOSE AGAR

supersedes media previously employed for the isolation and cultivation of the *Brucella*. This medium serves ideally for the primary or secondary isolation of *Brucella*, for the differentiation of species and for vaccine or antigen production. It is also recommended for use as the solid phase in the Castaneda technique.

THE DIFCO MANUAL, *NINTH EDITION*,
including descriptions of these media and their use, is available on request.

Specify DIFCO — the trade name of the pioneers in the research and development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
DETROIT 1, MICHIGAN